

# **A Text Book Reproduction in Farm Animals**

## **(THERIOGENOLOGY)**

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**VARGHESE PUBLISHING HOUSE**

**BOMBAY**

**1982**

Printed by S. V. Limaye at the India Printing Works, 9 Nagindas Master  
Road, Extension 1, Fort, Bombay, and published by K. M. Varghese,  
Varghese Publishing House, Hind Rajasthan Building,  
Dadar, Bombay 400 014.



Prof. Nils Lagerlof demonstrating a monster to the FAO/SIDA participants at the International Postgraduate Course on Animal Reproduction.

**Dedicated to the Memory of**  
**Professor Emeritus Nils Lagerlof**

Ph D , Med Dr (Stockholm),  
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## ***Preface***

Editors have very great pleasure in presenting this text book to the Under-Graduate and Post-Graduate students of the Veterinary, Animal Sciences and Agricultural faculties. This will also serve as a reference book to scientists and veterinary practitioners.

This text book is actually an outcome of the valuable teachings of Prof. Emeritus Nils Lagerlof, F.A.O. Expert, initial lead taken by Dr. P Bhattacharya of the Indian Veterinary Research Institute, Izatnagar and years of teaching and research experience of the authors in the field of Reproduction in Farm Animals, particularly under tropical conditions.

Most of the books written on the subject are by the eminent scientists from the West which no doubt serve as guidelines in teaching and research but their studies pertain to the researches based on the continental breeds of animals under agro-climatic conditions of the West. The needs of Veterinary students vary to a certain extent depending on the country, the type of environmental conditions and effects thereof on health, efficiency of reproduction and production levels especially in the dairy cattle, buffaloes, sheep and poultry. Necessity of a comprehensive text book was therefore felt since long by students in the tropical countries. A modest attempt is therefore being made in the production of this text book which deals with studies on the reproduction in cattle, buffaloes, horse, sheep, goat, swine, dog, cat, camel and also poultry.

The text book is divided in five parts. Part I deals with Obstetrics. Special emphasis has been given on comparative studies in different species of domestic animals. Eutokia and dystokia in farm animals have been dealt with exhaustively. Chapters on lethal factors and malformations are of great significance which will interest the readers.

Part II deals with Animal Gynaecology in which infective and non-infective conditions of genital tract affecting efficiency of reproduction in farm animals have been dealt with very precisely. The exhaustive information will be of great use to the field veterinarians. Chapters on corpus luteum, cystic ovarian degeneration, sexual health and udder health control have a great bearing on diagnosis and treatment of various reproductive disorders.

Part III is concerned with Andrological studies. Special emphasis has been laid on the andrological investigations and problems of infertility in the male domesticated animals.

Part IV deals with Seminology and Artificial Insemination. It is dealt with very exhaustively since breeding of cows and buffaloes by A.I. forms an integral part of the veterinarians' duties.

It is not possible to incorporate everything in a text book but since it is necessary to acquaint the students with the latest developments in science and to have deeper knowledge, Special Chapters have been introduced in Part V on, Mammalian egg and Transplantation of Fertilized ova, Inheritance of fertility and infertility, Nutrition in Relation to Reproduction, Sexual Behaviour in Domesticated Animals, effect of stress on reproduction, Neoplastic diseases of the Reproductive tract, and Surgical procedures in Obstetrical and Gynaec-Surgery. The comprehensive chapter on reproduction in Camel will be of great interest to the students in the tropics.

In this endeavour we are fortunate to have collaboration of the scientists from East and West and in this context the valuable contributions to this text-book by Drs. E. S. E. Hafez, A. F. Fraser, J. Becze, S. J. Miller, K. K. Vyas, R. P. S. Tyagi, Rama Mohana Rao, M. R. Marathe, R. C. Gupta, P. D. Sardeshpande, B. L. Purohit, D. S. Jadhav, C. K. Abdullakhan, P. E. Kulkarni, C. K. S. V. Raja, C. P. N. Iyer, K. S. Narasimhan, N. S. Tadpatrikar, M. B. Tatke, D. R. Pargaonkar, A. P. Dani, S. A. Sonawane, V. L. Deopurkar, P. K. Pareek and T. R. B. Nambudripad are gratefully acknowledged.

We sincerely hope that the text material will fulfil the long felt need of a comprehensive text-book on Reproduction in Farm Animals.

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EDITORS

15th August, 1982.

## Acknowledgements

Editors feel greatly indebted to late Emeritus Prof Nils Lagerlof and Dr P Bhattacharya for their valuable teachings guidance and encouragement which inspired them to undertake the heavy responsibilities concerned with the write up of this text book

Editors would like to express their profound regards to W L Williams F H A Marshall F Benesch J Richter R Gotze J Hammond G H Arthur, S J Roberts E S E Hafez H H Cole and P T Cupps L E McDonald, I Zuckerman T Mann R Zemjanis J A Laing E J Perry V A Rice E J Underwood and J P Maule for their valuable contributions in the field of Animal Reproduction which is a great source of information

Science of various States in India; Dairy Development Commissioners of various States in India; Food and Agricultural Organisations of the United Nations; Swedish International Development Authority, Sweden; Milk Marketing Board of England; Bhartiya Agro-Industries Foundation, Uralikanchan; Bombay Gowrakshak Mandali; Bombay Panjarpole; Gowshalas, Nasik, Bhojapur and Ahmednagar; Milk Federations from various States in India; Indian Dairy Research Institute, Karnal; Institute for Research in Reproduction, Bombay for having shared with the Authors their data, photographs and illustrations.

Special thanks are due to Prof. T. Mann, author of the book "Biochemistry of semen and of the male reproductive tract", published by M/s. Methuen & Co. Ltd., for having permitted to reproduce Table Nos. 10, 11, 16, 20, 25, 26, 28, 29 and 30.

Thanks are due to Prof. K. S. Deshpande and to Dr. D. G. Bhole, for the photographs on reproductive disorders in buffaloes and goats.

Our indebtedness in the production of this text book is very heavy and we gratefully acknowledge the valuable assistance rendered by the post-graduate students during the preparation of the manuscripts and profuse thanks are due to Drs. B. G. Mokashi; K. Rangiah; R. P. Nene; L. N. Purbey; P. P. Ponskshe; R. M. Bhandari; K. G. Gujarathi; A. B. Kulkarni; P. K. Amritkar; S. G. Zanwar; S. S. Jadhav; J. S. Patil; S. P. Dhingra and V. G. Phalak.

Thanks are also due to Dr. Krishnaji S. Gore; Mrs. Sudha Gore; Mrs. Snehaprabha Pradhan; Dr. (Mrs.) Prabha Bharucha; Dr. (Mrs.) Prabha Borwankar; Dr. G. M. Dhadphale; Dr. S. G. Dixit and Mr. A. A. Bhat for their valuable suggestions.

Editors feel grateful to Mr. N. V. Gunjekar, Managing Director AR-Ex Laboratories, Bombay; Mr. C. M. Ketkar, Agricultural Equipment Corporation, Pune and Dr. P. P. Jamkhedkar, Pfizer Limited, Bombay, for extending various types of aids in the production of this text-book.

Sincere thanks are due to Artist Mr. S. G. Bhurke of the Bombay Veterinary College for the photographs and diagrams and to Messrs. N. S. Wad; D. S. Nadkarni; Ashok Nabar and A. G. Mane for the typing work.

Co-operation rendered by Prof. S. R. Hattangadi; Prof. S. G. Kshirsagar; Prof. N. S. Deodhar; Prof. S. M. Ajinkya; Prof. V. S. Bhawe; Prof. V. L. Paranjape; Prof. S. M. Niphadkar; Prof. M. K. Shingatgeri; Prof. P. V. Naik; Prof. K. S. Deshpande; Prof. B. A. Kulkarni; Prof. M. V. Kulkarni; Prof. G. S. Koranne; Prof. P. M. Puntambekar; Prof. J. B. Khot; Prof. S. S. Mehendale and other staff members from various departments of the Bombay Veterinary College is gratefully acknowledged. Thanks are also due to Dr. S. K. Gupta, I.V.R.I. and Dr. R. V. Patil, U.A.S., Bangalore.

The book was held up in the manuscript form for years due to unavoidable circumstances. Members of our families Mrs. Sushila Sane; Mrs. Mandakini Luktuke; Mrs. Sumitra Deshpande; Mrs. Shreelekha Kaikini; Mrs. Meenakshi Velhankar; Mrs. Arati Hukeri; Mrs. Laxmi Kodagali; Mr. Abhay Palnitkar; Mrs. Ujawala Palnitkar; Mrs. Anuradha Kelkar and Miss Chaya Kale, patiently awaited for years to see the material in print. Their assistance, encouragement and co-operation is gratefully acknowledged.

It is a pleasant duty to acknowledge our indebtedness to Mr. K. M. Varghese of Varghese Publishing House and Mr. Anand Limaye of India Printing Works, Bombay and their staff for shouldering the responsibilities and meticulous attention provided by them in the production of this textbook

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PART I  
**OBSTETRICS**

## INTRODUCTION

The origin of the word "Obstetrics" is derived from the Latin word "Obstrix" meaning a midwife and "Obstare" meaning to stand before. It is also described as that branch of science which deals with the care of female during gestation, parturition and puerperium.

It is observed that the rise and progress of human midwifery is closely associated with the history of medicine in general. The followers of Hippocrates (400 B.C.) advocated the perforation of hydrocephalus, breaking of bones, extraction by hooks, turning of foetus

Louis XIV inviting a male doctor to attend on his Queen who was in labour.

Heinrich Van Deventer (1696) of Holland is aptly considered to be the father of modern human midwifery on account of his voluminous contribution to the science. Palfyn (1723) of Ghent was the first to demonstrate the use of forceps in obstetrical work.

During and after 18th century, the science of midwifery made greater strides at the hands of medical experts. Simpson (1847) used ether and chloroform for anaesthesia during labour.

trices in 1877. The two treatises by Williams (i) Veterinary Obstetrics and diseases of breeding animals and newborn (1943) and (ii) Diseases of genital organs (1943) are commendable. These volumes emphasise the views that dystokia and allied phenomena are predictable and preventable. The book "Veterinary Obstetrics and Zoo technique" published by Beeman in 1931 deals mainly with equine species. Another contribution is by Benesch in German language which was later translated in English by Wright in 1950 and further revised by Arthur in 1964. Veterinary Obstetrics and Genital diseases by Roberts (1956) and (1971) contained the review of recent literature. Considerable advances in obstetrics and gynaec surgery, surgical techniques, antibiotic therapy and supportive treatments have been developed during the recent years which have contributed a great deal for relief of several conditions.

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# Chapter I

## Obstetrical Anatomy

### PELVIS OF COW

Pelvic cavity is the posterior-most of the three large body cavities. It is bilaterally symmetrical and almost horizontal in domestic animals. Bony and ligamentous pelvis serves to contain, sustain and protect the urogenital system and the end parts of digestive tract. It also gives support for attachment of many muscles. It serves as a channel of abdominal cavity to the outside through which the foetus has to pass at the time of parturition. It is located at the end of vertebral column supported by two hind legs.

The lateral walls and floor of the pelvis is formed by two ossa coxae or innominate bones (hip bones) and the roof is formed by sacrum and first two or three caudal vertebrae. (Fig A-1)

gluteus muscles. It is crossed by a curved gluteal line which is parallel to the lateral border. Iliac surface which faces forwards is situated lateral to ilio-pectineal line and gives attachment to iliacus muscle. Pelvic surface is smooth, concave and forms lateral boundary of pelvis. On rectal and vaginal palpation it can be easily detected. Obturator nerve crosses its surface, injury to which may occur at the time of parturition leading to obturator paralysis. At its upper part is a triangular auricular facet for articulation with sacrum.



skeleton. The lower angle joins the other two bones — ischium and pubis at the cotyloid cavity.

*Ischium*: Next in size to ileum, ischium forms the posterior part of the pelvic floor. It is thin, quadrilateral and curved.

Pelvic surface of ischium is smooth and concave from side to side and from before backwards in form of a basin. Ventral surface is curved, rough and serves for attachment of the adductor muscles of the thigh.

Anterior border is thick forming posterior margin of obturator foramen. Posterior border is curved forming ischial arch which is narrow and deep. Lateral border is smooth, concave and forms lower margin (lip) of lesser sacro-sciatic foramen. Medial borders join to form ischio-pubic symphysis. They bear a ridge ventrally which fades out posteriorly.

Antero-lateral angle is large and joins other two bones at acetabulum. It is grooved medially through which the obturator vessels pass. Anterio-medial angle joins posterior angle of pubis. Postero-lateral angle called tuber ischii is a thick trifid process and gives attachment to biceps femoris and semitendinosus muscles. Postero-medial angle forms the summit of ischial arch.

*Pubis*: Pubis is the smallest of the three segments of hip bone and forms the anterior portion of the floor of pelvis. It is wide and triangular in shape.

Pelvic surface of pubis is smooth and concave in adult animals, convex in young ones. Ventral surface is rough and presents a large sub-pubic groove which does not extend upto the acetabulum. It lodges a large subpubic vein.

Anterior border (pecten ossis pubis) is thin, uneven and forms anterior margin of the pelvic inlet. The tuberculum pubicum is situated at its central part and from which prepubic tendon arises.

At times this may project in the cavity and lead to laceration of foetus during dystokia. The border laterally bears the ilio-pectineal eminences. Posterior border is thick, concave and forms anterior margin of obturator foramen. Medial borders join at ischio-pubic symphysis.

Lateral angle is large, thick and joins other two bones at acetabulum. Medial angle joins its counterpart. Posterior angle fuses with antero-medial angle of ischium and forms medial margin of obturator foramen.

*Acetabulum*: It is a cotyloid cavity meant for articulation with the head of femur. It faces ventrolaterally and consists of articular and non-articular parts. Its rim is rounded and marked by anteriomedial and posteriomedial notches which are often converted into foramina by ligaments in fresh state. Two depressions in front of it give attachment to rectus femoris muscle. Two-fifth (40%) of the cavity is formed by ileum, one-half (50%) by ischium and one-tenth (10%) by pubis.

*Obturator foramen*: This is situated between ischium and pubis. It is large, elliptical in outline and its medial margin is thin and sharp. Anterio-lateral margin is grooved through which obturator vessels and nerves pass. In fresh state, it is covered by thin obturator membrane and obturator internus muscle.

### PELVIS OF MARE (Fig. 1a)

*Ilium*: The two ilia are very large and extensive. Gluteal line is not very distinct and parallel to the lateral border as in the cow but it starts from middle of the border and runs towards tuber coxae. The tuber coxae is bifid, wide in the middle and narrow at either ends.

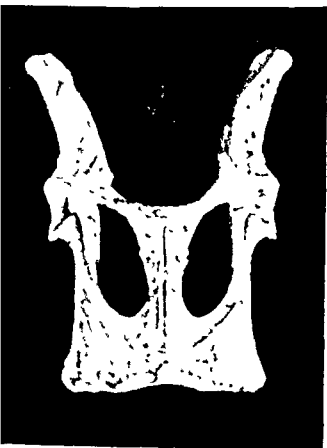
*Ischium*: It is thicker and not so curved. Its dorsal surface is flat. Tuber ischii is bifid and thick. Ischial arch is wide. Ventral ridge at ischio-pubic symphysis is absent.



Fig A-1 Pelvis of Cow



Fig 1a Pelvis of Mare



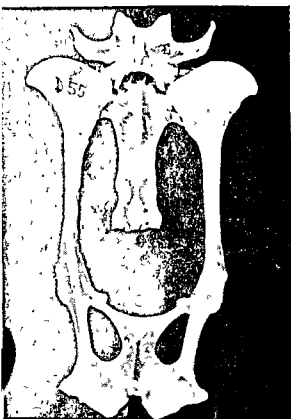


Fig. 1d. Pelvis of Doe (She Goat)



Fig. 1f. Pelvis of Fowl

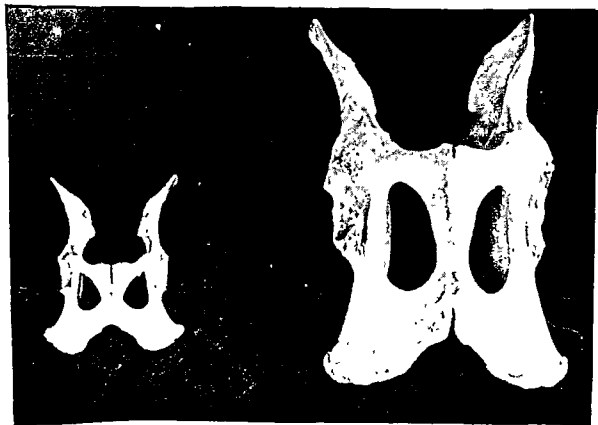


Fig. 1e. Pelvis of Cat and Bitch

**Pubis:** It is comparatively narrow. The sub-pubic groove is large and leads to acetabulum. Centrally it contains a vein and its lateral part contains the accessory ligament of hip joint. Tuberculum pubicum is large.

**Acetabulum:** It is large and its medial part presents acetabular notch which is converted into a foramen in fresh state and through which pubo-femoral or accessory ligament passes to the head of femur.

**Obturator foramen:** The large and oval obturator foramen has a thicker medial border.

### PELVIS OF BUFFALO (Fig. 2, 3)

**Ilium:** The gluteal line is directed midway between ischiatic spine and acetabulum which fades out below and does not join the ischiatic spine. The grooves for iliolumbar vessels are not distinct. The triangular area for muscular attachment below the tuber coxae is larger than that in the cow.

**Ischium:** Superior ischiatic spine is high, thin, irregularly sharp and fragile. Ischial arch may be either rounded or acute angled. The ridge on the ventral aspect of ischio-pubic symphysis is prominent at the ischial arch and fades out in the centre.

**Pubis:** The fusion between two halves is not usually complete. The sub-pubic groove is not very distinct. The pecten ossis pubis extends centrally in form of a hook that is directed downwards, forwards and upwards.

**Acetabulum:** Antero-medial acetabular notch is smaller than postero-medial notch. The distance between acetabulum and tuber coxae is greater by about 8-10 cm than the distance between acetabulum and tuber ischii (Hadi and Sane 1955). In cows these are nearly equal.

### PELVIS OF EWE AND GOAT

(Fig. 1 c, d)

It resembles that in the cow except in size.

**Ilium:** It is longer, more horizontal than in the cow and superior ischiatic spine is less developed. Tuber sacrale is pointed.

**Ischium:** Tuber ischii is flattened, everted and bears a long blunt pointed process.

**Pubis:** Pecten is thin and sharp.

### PELVIS OF SOW (Fig. 1 b)

It is longer and narrower.

**Ilium:** Crest of ilium is convex. Gluteal line is very distinct which divides gluteal surface into two concave areas. Superior ischiatic spine is large and prominent.

**Ischium:** The muscular ridges on lateral aspect of superior ischiatic spine are very prominent.

**Pubis:** It is narrow and thick. The psoas tubercle is very prominent.

**Acetabulum:** It is placed further back than in the cow and the rim of acetabulum is thicker.

### PELVIS OF BITCH AND CAT

(Fig. 1 e)

**Ilium:** Ilium is vertical and parallel to median plane. Gluteal surface is depressed. Pelvic surface is flat. The auricular facets face directly inwards. The crest of ileum is strongly convex. Tuber coxae is rounded. Shaft of ileum bears a ventro-lateral crest which terminates at a tuberosity in front of acetabulum.

and greater sacro-sciatic notch is shallow.

*Pubis*: The sub-pubic groove is absent.

*Acetabulum*: The fossa acetabuli is deep and bounded medially by a flat thin plate of bone which is so thin as to be translucent.

*Obturator foramen*: It is roughly triangular with rounded angles.

### SACRUM OF COW

This bone is formed by fusion of five sacral vertebrae and forms the roof of pelvis. It is triangular in outline with long axis curved. The dorsal surface presents the median sacral crest formed by fusion of sacral spines. Lateral crests are formed by fusion of articular processes and lateral to these are four pairs of dorsal sacral foramina.

Ventral surface is concave and marked by four distinct transverse lines which vary with breed, sex, age and individual. Centrally there is a groove for passage of median sacral artery. Four pairs of ventral sacral foramina larger than the dorsal series are present at the end of linea transversae. The lumbo-sacral nerves emerging through these give rise to obturator and gluteal nerves which are likely to be injured, if there is dystokia.

Anteriorly, the base of sacrum articulates with last lumbar vertebra. The wing of sacrum presents triangular auricular facet for articulation with ileum below which the sacro-sciatic ligament is attached. Promontory or sacro-vertebral angle is not prominent compared to other species. Apex is smaller and triangular. The lateral borders are thin, sharp and curve downwards.

### SACRUM OF BUFFALO

It resembles that of the cow. Sulcus vasculosus is absent. Promontory is more flattened dorso-ventrally. The

fifth sacral vertebra is not usually fused with the fourth.

### SACRUM OF MARE

Sacrum consists of five bones. It is shorter and less arched. There is no median sacral crest but sacral spines are separate. Ventral surface is flat and smooth. Wing of sacrum presents two elongated facets for articulation with transverse processes of the last lumbar vertebra. The auricular facets are also elongated.

### SACRUM OF EWE AND GOAT

It resembles that of the cow and consists of four bones only. Sulcus vasculosus is absent. Transverse processes of the last segment are distinct and outstanding.

### SACRUM OF SOW

Sacrum is less curved and consists of four segments. Inter-annular spaces are wide. Linea transversae are distinct.

### SACRUM OF BITCH AND CAT

Sacrum consists of three segments only. It is wide, quadrangular and wings are prismatic. Auricular facets face outwards. Median sacral crest has two notches. Two pairs of dorsal and ventral sacral foramina are present.

### COCYGEAL VERTEBRAE

The first two or three coccygeal vertebrae enter into formation of the roof of pelvic cavity. The number varies in different animals and may be 20-22 in cow, 15-18 in mare, 7-8 in goat, 3-24 in ewe, 15-18 in sow and 16-24 in bitch. First few are the typical vertebrae with all the processes in a suppressed form.

### ARTICULATIONS

Four joints are involved in pelvis. They are:

- (i) Sacro-lumbar articulation.
- (ii) Sacro-iliac articulation.

- (iii) Ischio-pubic symphysis.
- (iv) Sacro-coccygeal articulation.

#### (i) Sacro-lumbar joint

This joint is formed between the sacrum and last lumbar vertebra which is further strengthened by ligamentum flavum. Greater mobility of this joint favours change of direction of pelvis during parturition. Incomplete luxation of this joint may lead to hindrance at parturition.

#### (ii) Sacro-iliac joint

This is a gliding joint formed between auricular surfaces of sacrum and ilium. Sacrum fits like a wedge between two ilia. Movements are very limited. Four ligaments are accessory to this joint. They are:

- (a) Dorsal sacro-iliac ligament.
- (b) Sacro-sciatic ligament.
- (c) Lateral sacro-iliac ligament.
- (d) Ilio-lumbar ligament.

These bind the pelvis to the vertebral column.

*The dorsal sacro-iliac ligaments:* These extend from tuber sacrale to summits of sacral spines in the form of a band.

*Ilio-lumbar ligament:* Ilio-lumbar ligament extends from lumbar transverse processes to the ventral surface of ilium.

*Lateral sacro-iliac ligament:* It is triangular and extends from tuber sacrale to lateral border of sacrum.

sacro-sciatic notch. Here it forms the great sacro-sciatic foramen meant for the passage of gluteal vessels and sciatic nerves, compression of which may lead to cramps and paralysis of hind limbs during parturition.

Posterior border is fused with semi-membranosus muscle.

Medial surface is covered by peritoneum and is in relation to genital organs. Lateral surface is covered by gluteus, biceps femoris, semitendinosus muscles and sciatic nerve.

#### (iii) Ischio-pubic symphysis

It is situated between two ossa coxae at mid-ventral line. Movements of this joint are very limited. A crest may develop on pelvic surface of the joint which may interfere with manipulations during dystokia.

#### (iv) Sacro-coccygeal articulation

Fusion of first coccygeal with last sacral vertebra may complicate parturition due to rigidity.

### COMPARATIVE ARTHROLOGY

The pelvic joints in other domestic animals present no striking dissimilarities except in mare. In this species, inter-transverse joints are present between the last lumbar vertebra and wing of sacrum. Such joints are absent in buffalo, ewe, goat, sow, bitch and cat.

inlet is entirely bony, rigid and continuous with abdominal cavity but the pelvic outlet is most dilatable. The prepubic tendon of the abdominal muscles is inserted on the cranial border of pubis thereby forming a prominence on pelvic floor. The calf has to overcome this step while entering the pelvic cavity.

### **PELVIC CAVITY OF THE BUFFALO**

As in the cow, 28 muscles either take origin or are inserted on the pelvis. In addition, ventral sacro-coccygeus muscle consists of two heads instead of one as in the cow (Hadi and Sane, 1965).

### **PELVIC CAVITY OF THE MARE**

Pelvic cavity is not compressed from side to side and the walls are not parallel. It is shorter. There is a depression on the dorsal surface of pubis in which previously emptied bladder can rest during foaling. Roof is less arched.

### **PELVIC CAVITY OF THE EWE AND GOATS**

It resembles that in the cow except in size. Symphysis is linear. Pelvic axis inclines downwards caudally.

### **PELVIC CAVITY OF THE SOW**

Pelvis is very spacious and its axis inclines downwards caudally. Promontory is very distinct.

### **PELVIC CAVITY OF BITCH AND CAT**

Pelvis is very oblique. The promontory is distinct. Floor is concave, narrow in front than behind where it is flat. Between the superior ischiatic spines at the acetabula, the distance is very small where the foetal head may get arrested during parturition. Obturator foramen in the cat is larger.

## **DIFFERENCES IN PELVIS ACCORDING TO SEX**

On account of the functional differences, the pelves of male and female do differ. However, these differences can not be considered as authentic proof for identification of sex by looking at the pelvic bone alone. The pelvis of animal that has been castrated early in life presents feminine characteristics. In the female, pelvis is larger and more roomy; pelvic inlet is larger and more oblique; the ilio-pectineal lines are further apart; conjugate and transverse diameters are greater; the floor is concave and pelvic outlet is larger. Ischial arch is more wide and greater and lesser sacro-sciatic notches are broader and shallower. The sacrum is broad, long, more arched and presents indistinct transverse lines than those in the male. The pelvic bones of female are thinner and lighter than in the male.

### **PELVIMETRY-CAPACITY OF PELVIS**

Chauveau and Arloing (1891) defined pelvimetry as that branch of Obstetrics which deals with the diameters of pelvis. Pelvimetry has attained greater significance in human obstetrics since the pelvic abnormalities are more common. This is on account of the fact that the posture being vertical, the entire body weight is thrown on pelvis and therefore it is liable to be deformed. In the quadrupeds, the pelvis does not bear direct body weight and in addition it is supported by two hind limbs at the acetabulum. The pelvic deformities due to pressure are therefore very rare. Thus the pelvis plays a very passive role in Veterinary obstetrics and the veterinarian is rarely consulted for pelvimetry.

The study of pelvimetry is warranted in the days of selective breeding and cross breeding since the relationship between various diameters allow one to form an idea of shape, size, position of



Fig 2 Buffalo Pelvis — Dorsal view



Fig 3 Buffalo Pelvis — Ventral view



← Fig 4(b) Camel Pelvis — Dorsal view

Fig 4(a) Camel Pelvis — Ventral view





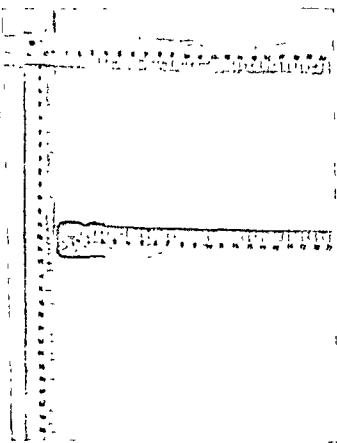


Fig. 6 Pelvimeter

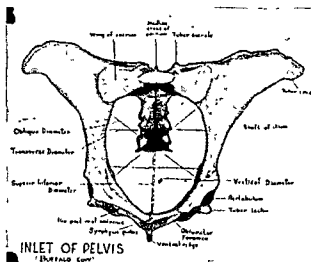


Fig. 5. Diameters of Pelvic inlet (Buffaloe)

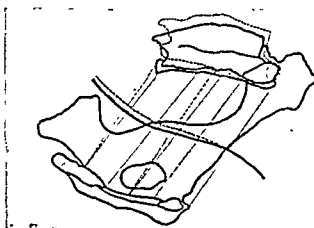


Fig. 7. Pelvic Axis (Normal and during parturition) (Buffaloe)

Table 1  
AVERAGE DIAMETERS OF PELVIS IN DIFFERENT SPECIES

Height	Pelvic Diameters (Cms)						Authors
	Superior		Vertical		Transverse		
	Inlet	Outlet	Inlet	Outlet	Inlet	Outlet	
Medium size	22.10 22.56-24.13	— 22.86	20.07 —	20.07 —	18.03 17.14-19.68	19.05 22.86	Rainard (1845) Baumeister & Rueff, cited by Craig (1930) Carsten Harms (1875) Saint Cyr (1875) De Bruin (1901) Sisson & Grossman (1955) Roberts (1956)
(M msl)	24.42 (21.08-28.96)	—	22.63 (19.05-24.13)	20.55 (16.51-23.62)	18.67 (16.00-20.57)	18.67 (16.00-20.32)	Luigi and Sano (1965)
15 Hunch	24.13	—	23.47	17.19	23.47-24.13	19.05	Craig (1930)
14 Hunch	23.47-24.13	—	22.10	15.24-16.08	22.10-22.68	17.02-17.78	
12 Hunch	21.08-22.10	—	20.07	10.92-11.94	19.05-20.07	12.95-13.97	
	22.86-24.43	22.86-23.43	—	—	27.94-31.75	22.86	Baumeister & Rueff cited by Craig (1930)
	24.13	19.05	—	—	23.37	16.51	Carsten Harms (1875)
	22.86	17.78	—	—	23.37	19.05	Arling (1868) Cited by Craig (1930)
	17.14	—	—	—	20.57	—	Saint Cyr (1875)
Medium	23.08	—	14.99	11.94	11.94	9.91	Craig (1930)
Oblique	11.94	—	6.13	8.93	7.89	6.60	Craig (1930)
St. Item	12.08	—	6.98	6.98	5.92	6.98	Craig (1930)
Trans	10.16	—	8.13	6.10	8.13	10.16	Craig (1930)
Longitudinal measured to full conjugality	4.93	—	5.13	5.93	5.03	5.03	
Small	4.08	—	4.08	4.93	3.03-4.06	4.06	
Oblique	6.53	—	4.98	5.93	3.03	4.06	

pelvis and the canal. It helps in culling undesired type of animals with narrow defective pelvis.

The term diameter in pelvimetry refers to distances between certain parts in pelvic cavity. These vary in different animals. There are five important diameters of pelvic inlet.

### Diameters of Pelvic inlet

- (1) Superio-inferior or sacro-public or conjugate diameter is the distance between promontory and pubis.
- (2) Superio-transverse or bis-iliac diameter is measured between upper third of pelvic cavity.
- (3) Inferio-transverse or bis-iliac diameter is measured in lower fourth of pelvic cavity between the two psoas tubercles.
- (4) Oblique or ilio-sacral or sacro-iliac diameter pass from sacro-iliac joint, through the centre of pelvic cavity to the psoas tubercle of opposite side.
- (5) Vertical diameter is the perpendicular distance between pubis and third/fourth sacral vertebra (Fig. 5).

### Diameters of pelvic outlet

- (1) Superio-inferior diameter is taken between the body of the first coccygeal vertebra and the summit of the ischial arch.
- (2) Transverse diameter is measured between the two tuber ischii.

Pelvimetry in living animal may be carried out in two ways viz. (1) External pelvimetry and (2) Internal pelvimetry.

### 1. EXTERNAL PELVIMETRY

This is arrived at by determining the diameters of pelvis from certain body measurements. It is necessary to ascertain:

- (i) The distance between two angles of haunch ..... A
- (ii) The distance between two ischial tuberosities ..... B

(iii) The height from hip-joint to summit of croup ..... C

For measuring A, B and C, straight pieces of wood or pelvimeter (Fig. 6) is used:

Transverse diameter of outlet:

$$1/4 A + B = X$$

Superio-inferior diameter of outlet:

$$3/4 C = Y$$

Transverse diameter of inlet:

$$12.2$$

$$\frac{\text{---}}{\text{---}} \times X$$

$$10$$

Sacro-public diameter of inlet:

$$13/10 \times Y$$

Hadi (1965) reported that various external and internal measurements of Murrah buffalo pelvis are related to a greater or lesser degree and established a regression equation.

### 2. INTERNAL PELVIMETRY

It is done by direct manual examination of the interior of pelvis on rectal and vaginal examination. The distances between various parts of pelvis can be approximately determined by calibrating the palm.

*Axis of Pelvis:* It is an imaginary line passing through the centres of pelvic inlet, cavity and outlet. It is almost rectilinear and directed backwards. (Fig. 7)

Pelvic measurements are presented in Table 1.

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# Chapter 2

## Reproductive Organs in the Female

The reproductive organs in the Female are divided in three parts.

- (a) Primary sex organs or gonads i.e. the ovaries.
- (b) Reproductive tract consisting of fallopian tubes, uterus and vagina.
- (c) External genitalia which consist of vestibule, clitoris, labia and glands which open in the posterior part of vagina.

A brief account concerned with the embryology, morphology, comparative anatomy, cyclical changes and functions of the female reproductive organs in-

cluding mammary glands in the farm animals are dealt with in the present chapter (Fig. 10, 11, 12, 13).

### EMBRYONIC DEVELOPMENT

Prior to sex differentiation, the basic structures of reproductive organs in the male and female are essentially the same. The genitalia is composed of two undifferentiated gonads, two pairs of ducts and an urogenital sinus. The gonads develop from the genital ridge on dorsal side of the abdominal cavity. Its anterior and posterior ends degenerate while the middle portion enlarges and is invaded by a group of large granular yolk sac cells. In the female development, two invasions occur during the course, the initial one being abortive, whereas the second one results into the

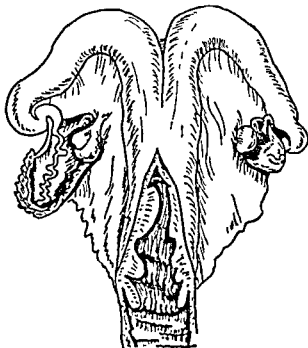


Fig. 10: Uterus and ovary of cow.

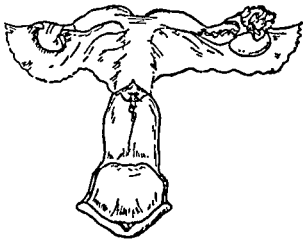


Fig. 12: Reproductive organs of mare.

formation of sex cords which later break up into primordial germ cells known as oogonia. These sex cords are called as "Pflüger cords" and form separate follicles. With the appearance of secondary sex cords, those formed during first invasion do not grow further and disappear in medulla of the ovary (Gruenwald, 1942). The gonads contain the cell complement which helps to develop either into a male or female. However, subsequent differentiation depends upon both the genotype and the external and internal environment. Before the completion of the migration of germ cells, a marked proliferation of the germinal epithelium in the gonads results in the formation of sex cords. In the female, they are termed as medullary cords and in the male as seminiferous tubules. From these cords are developed the primary vesicles which later form the Graafian follicles. Although the sex is determined at the time of fertilization, sex cannot be differentiated until about 7th week of development.

The Wolffian and Mullerian ducts are located on either side of mid-line, the Mullerian ducts being nearer to the centre. In the female, the Mullerian ducts grow and develop into the female duct system, whereas Wolffian duct regresses and remains rudimentary as vestigial structure. Posterior ends of the Mullerian duct from either side come close together dilate and then fuse to form the common body of uterus, cervix and anterior portion of vagina. In the female, the Wolffian duct persists as Gartner's canals.

side. Phallus becomes the clitoris in female and is a homologue of the penis in the male. The urogenital sinus forms the lips of vulva and posterior part of vagina. External genitalia are fully formed by about three months of gestation.

The homology of the reproductive organs is shown in Table 2.

## OVARY

### Morphology of the Ovary

The ovaries of the cow are situated near middle of the lateral margin of pelvic inlet in front of external iliac artery. They are intrapelvic and can be palpated per rectum near the anterior extremity of uterine horn situated about 40-45 cm. from vulval opening. They are almond shaped, firm, ovoid bodies, flattened on both sides. The size, form and location of ovary varies in different animals according to breeds, species, age and individual.

Each ovary is about 4 cm. in length, 1.25 cm. in width and its weight varies between 7 to 15 gm. Right ovary is slightly larger than the left. The size and weight varies according to the stage of oestrous cycle, number of follicles and types of functional and regressed corpora lutea. By the onset of puberty, the ovary increases 4-7 times its weight at birth. Ovulation occurs from any part of the exposed surface of the ovary wherever follicles develop. There is no ovulation fossa in the cow.

Table 2  
HOMOLOGIES OF FEMALE AND MALE REPRODUCTIVE SYSTEM

(Nalbandov, 1969)		
Indifferent	Female	Male
Gonad	Ovary Rete ovarii*	Testis Rete testis
Mesonephric tubules	Epoophoron* Paroophoron*	Vas efferens Paradidymis* Vas aberrans*
Mesonephric duct	Duct part of Epoophoron* (Gartner's duct)*	Epididymis Vas deferens Ejaculatory duct. Seminal vesicle Appendix of epididymis*
Mullerian duct	Appendage of the ovary* (hydatid) Fimbria of oviduct Oviduct. Uterus. Vagina (all or part?)	Appendage of testis*  Prostatic utricle (uterus masculinus)*
Urogenital sinus	Urethra Vestibule Vagina (in part?) Vestibular glands (Bartholins glands)  Para urethral glands*	Prostatic, membranous and cavernous urethra  Bulbo urethral glands (Cowper's glands)  Prostate
Genital tubercle	Glans clitoris Corpus clitoridis	Glans penis Corpus penis.
Urethral folds	Labia (minora)	Raphae of scrotum and penis.
Labioscrotal swellings	Labia (majora)	Scrotum.

\* Rudimentary. Words in parentheses are synonyms.

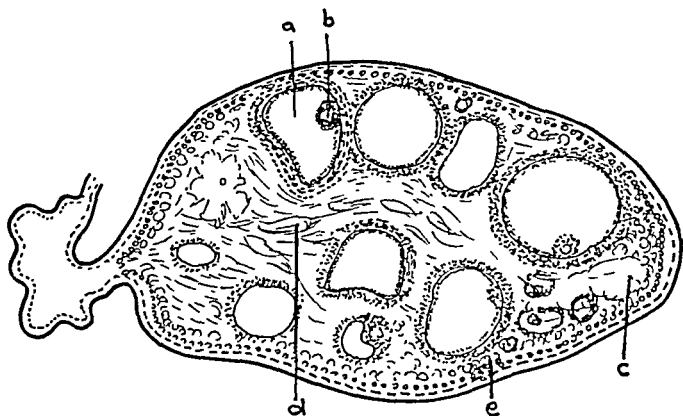


Fig 8: Diagrammatic sketch of ovary showing: (a) Graafian follicle, (b) Oocyte in cumulus oophorus, (c) Corpus luteum, (d) Medullary substance, (e) Primary follicle

smooth muscles and lymphatics (Fig. 8). The arteries take a spiral course and are known as helicine arteries. The cortex of ovary contains follicles, primordial follicles and atretic follicles. It serves as a site both for egg formation and production of hormones. The dense outer layer of cortex which consists of parallel bundles of fibroblasts is termed tunica albuginea. There is a layer of flattened cells on the surface of ovary known as germinal epithelium. In the ovary the germinal epithelium is located in the outer surface whereas in testicles it is within the gland.

are (a) Primary, (b) Growing, and (c) Mature.

#### (a) PRIMARY FOLLICLES, PRIMORDIAL FOLLICLES OR OOGONIUM

These are smallest in size and persist for many years in resting form in the ovarian cortex showing no signs of growth whatsoever. Primordial follicle consists of a oocyte  $20-30\mu$  in diameter surrounded by a squamous or squamous cuboidal follicular epithelium.



measure 40-60  $\mu$  whereas the latter may be 40-80  $\mu$  in size. As growth proceeds, the zona pellucida becomes more distinct, the follicle is separated from the stroma by a layer of membrana propria and it is displaced towards the centre of ovary.

### (c) GRAAFIAN FOLLICLES OR MATURE FOLLICLE

This term is applied to only those follicles which show antrum or cavity (Fig. 9). The oocyte has a diameter of 80-120  $\mu$ . It is surrounded by a thick capsule about 4-6  $\mu$  in thickness of Zona-pellucida with radial streaks. The granulosa layer consists of 6-10 layers of cells depending upon the stage of development. Differentiation of theca layer into externa and interna is first distinguishable in Graafian follicles. The mitotic activity is greatest during early stages which later on decreases until ovulation. The antrum is lined by many layers of follicular cells called as membrana granulosa and the fluid content termed as liquor folliculi which has a high concentration of proteins and oestrogens. The mature Graafian follicle varies in diameter in different species. Its diameter varies between 12 to 19 mm. in cows, 5 to 10 mm.

in buffaloes, 5 to 10 mm. in ewes, 8 to 12 mm. in sow and 25 to 70 mm. in mare, 2 mm. in bitch and cat and 9-12 mm. in woman. It protrudes above the surface of ovary like a blister and is thin walled. On microscopic examination of the Graafian follicle, the central cavity or the antrum is lined by the cells of membrana granulosa situated on a basement membrane called membrana propria. The next layer consists of the inner vascular layer, the tunica interna. At one end of the cavity is a stalk of cells, called cumulus oophorus or discus proligerus, which surrounds the egg and protrudes into the antrum. The egg itself is surrounded by a vitelline membrane and perivitelline space.

The number of Graafian follicles that develop during each oestrous period depends upon the species. It is also governed by the hereditary, hormonal and environmental influences. Usually only one follicle develops in the cow, buffalo and mare during each oestrous cycle whereas the remaining follicles regress and become atretic. In the sow 10 to 25 follicles mature during each cycle and 1 to 3 in sheep depending upon the breed, age, season and plane of nutrition.

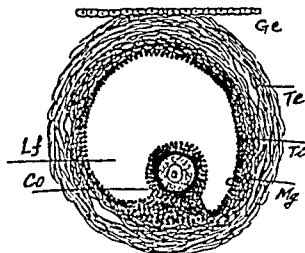


Fig. 9: Diagram of a Graafian follicle showing germinal epithelium (Ge), Theca externa (Te), Theca interna (Ti), Membrana granulosa (Mg), Cumulus oophorus (Co), Liquor folliculi (Lf).

### Blood Supply

The ovarian artery, a branch of utero-ovarian artery supplies blood to ovary. The venous blood is drained by ovarian veins.

Lymphatics are numerous and they pass to the internal inguinal lymph glands. The autonomic nerve supply comes from ovarian plexus arising from renal and inter-mesenteric plexuses.

### Ovary of the Buffalo

The ovaries of buffalo are elongated, more tightly secured and resemble those of cows. Details of ovarian biometry are presented in Table 3.

Table 3  
BIOMETRY OF BUFFALO OVARIES

Reference	Sane et al, 1964	Sane et al, 1965	Kaikini, 1974	Kodagali et al, 1971
Breeds	Murrah	Jaffri	Berari (Nagpuri)	Surti
No. of observations	877	214	966	330
Left ovary Length (cm.)	2.97 $\pm$ 0.02	3.13 $\pm$ 0.04	2.58 $\pm$ 0.56	2.25
Width (cm.)	1.37 $\pm$ 0.01	1.44 $\pm$ 0.03	0.90 $\pm$ 0.43	1.23
Thickness (cm.)	1.13 $\pm$ 0.01	1.15 $\pm$ 0.02	1.42 $\pm$ 0.51	1.20
Weight (gm.)	3.66 $\pm$ 0.06	3.87 $\pm$ 1.60	2.36 $\pm$ 2.15	2.50
Right Ovary Length (cm.)	2.91 $\pm$ 0.02	3.12 $\pm$ 0.04	2.42 $\pm$ 0.58	2.08
Width (cm.)	1.31 $\pm$ 0.09	1.49 $\pm$ 0.03	0.91 $\pm$ 0.32	1.20
Thickness (cm.)	1.17 $\pm$ 0.01	1.19 $\pm$ 0.08	1.48 $\pm$ 0.50	1.22
Weight (gm.)	3.81 $\pm$ 0.06	4.01 $\pm$ 1.53	2.28 $\pm$ 1.43	2.24

### Ovary of the Mare

Ovaries of mare are smooth, bean shaped, ovoid reniform bodies with firm consistency. These measure 8-10 cm. in length, 2.5 to 4.0 cm. in width and 70-105 gm. in weight. They are suspended in the sublumbar region by the mesovarian ligament below the transverse process of the 4th and 5th lumbar vertebrae just behind the kidneys. They are located 5 to 8 cm. dorso-lateral to uterine horns just in front and below the level of external angle of ilium. These are joined to the horns by distinct band of utero ovarian ligaments.

Each ovary presents a notch or hilum called ovulation fossa on its

### Ovaries of ewe and goat

The ovaries are almond shaped and 1.25 cm. or more in length. The measurements of right and left ovary as reported by Puranik and Kaikini (1968) are as under:

	Right ovary	Left ovary
Length (cm.)	1.61	1.64
Width (cm.)	1.13	1.09
Thickness (cm.)	0.77	0.76
Weight (gm.)	1.09	1.06
No. of observations	219	219

In the Doe the right ovary is more active than the left (Achuthan Kutty and Raja, 1971)

completely concealed in the bursa ovarii by mesosalpinx.

Table 4  
BIOMETRY OF SWINE OVARIES

	Zaki <i>et al</i> , 1967	Unpublished data Velhankar <i>et al</i> , 1980
Left :		
Length (cm.)	$2.49 \pm 0.11$	$2.96 \pm 0.16$
Width (cm.)	$1.43 \pm 0.05$	$1.71 \pm 0.05$
Thickness (cm.)	$1.57 \pm 0.03$	$2.23 \pm 0.13$
Weight (gm.)	$3.20 \pm 0.33$	$5.61 \pm 2.55$
No. of CL	$6.3 \pm 0.38$	$7.25 \pm 2.90$
Right:		
Length (cm.)	$2.32 \pm 0.07$	$2.92 \pm 0.10$
Width (cm.)	$1.12 \pm 0.04$	$1.46 \pm 0.42$
Thickness (cm.)	$1.70 \pm 0.04$	$1.89 \pm 0.09$
Weight (gm.)	$3.11 \pm 0.26$	$4.56 \pm 0.45$
No. of CL	$5.40 \pm 0.30$	$6.64 \pm 0.65$

#### Ovaries of the bitch

They are small, oval, elongated flattened bodies measuring less than 2.5 cm. in length and covered by a bursa ovarii which has a slit ventrally. The bursa usually contains adipose tissue. No distinct hilus is present. The ovaries are situated near the caudal pole of kidneys, opposite 3rd/4th lumbar vertebra between last rib and the crest of ilium.

#### Ovaries of the cat

They are similar to those of bitch and are suspended more ventrally in the abdominal cavity. They measure about 2.5 cm. in diameter. The ovarian bursa lacks in fat.

#### FALLOPIAN TUBES — COW

These tubes were described by Fallopius in 16th century and hence are named after him as fallopian tubes.

These are also called oviducts, salpinx or uterine tubes. The fallopian tubes are paired tubes which convey ova from ovaries to the uterus. In farm animals the ovaries are located in the open ovarian bursa. In farm animals, the bursa is pouch like. It consists of a thin peritoneal fold of mesosalpinx which is attached to the suspended loop at the upper portion of salpinx. In cow, ewe and goat the bursa is spacious and open. It is 4-6 cm. deep and 8-12 cm. wide in the cow.

The fallopian tubes are divided into three parts : (a) infundibulum with its fimbriated end, (b) ampulla, and (c) isthmus.

Length and degree of coiling differs from species to species. In the cow, the length of fallopian tube is about 18-25 cm. The width gradually decreases posteriorly being 4-8 mm. at the ovarian end and 2-3 mm. at the uterine end. The peritoneal opening of the oviduct adjacent to ovary is a funnel shaped dilated structure known as infundibulum. The size of fimbria varies with the species and age of the female. The surface area is 20 to 30 sq. cm. in the cow. In the centre of fimbria is the opening called ostium tubae abdominale. The fimbria is a free structure except for one point at dorsal pole of the ovary. This enables the fimbria to come close to ovary during ovulation to catch the viable ovum released from the ruptured follicle.

Next to fimbria is the ampullar part of oviduct. Isthmus is the constricted part next to ampulla which lies close to the uterus. The significance of ampulla and isthmus junction is not known. The isthmus is connected to the uterine cornua by an opening called ostium

tubae uterinae. A marked flexure at the uterotubal junction is observed during oestrus in cows and ewes (Hafez, 1968). The fallopian tubes can be palpated per rectum. The procedure for examination is as under:

The fingers are bent and slid under the mesovarium into the bursa. The bursa is exposed by spreading the fingers and hooked on to the thumb and lifted upwards. It should be possible to introduce 2-3 fingers easily into the normal bursa. The oviduct is then palpable as a thin cord like structure lying on the tips of fingers.

### Histology

The wall of the oviduct consists of three layers, viz., outer serosa, middle muscularis and innermost mucosa. The type of mucosa and the muscular layer varies in different parts of the oviduct. The mucous membrane is thrown into many primary and secondary folds. They are few in isthmus and abundant in ampulla. In mares and sows, they are much more prominent than in cows and ewes. In the mare, the secondary folds may be as high as sixty. The mucosa layer consists of lining epithelium, the muscle fibres and connective tissue which contains blood and lymph vessels.

The surface epithelium is of ciliated columnar type at the fimbria whereas few such cells are present near the uterine end. These cilia vibrate to wards uterine end. In addition to these, aciliated columnar secretory cells and thin columnar rod shaped cells

The muscular layer consists of inner circular or spiral muscle fibres and an outer longitudinal fibres. The thickness of this musculature increases from ovarian to the uterine end. The serous coat consists of connective tissue and is covered by an outer layer formed from peritoneum.

The fimbria contains a net work of muscle fibres and large blood vessels. It is composed of erectile tissue. During ovulation, these blood vessels get filled with blood which causes enlargement and tumefaction of the fimbria. Together with the contractions of the muscles, this tumefaction enables the ostium to come close to the ovary.

### Function

Following ovulation, the ovum is conveyed from the fimbriated end to the oviduct. Capacitation of the sperm, fertilization and early cleavage of the embryo occur in the oviduct. The ciliary action and the muscular contractions regulated by the ovarian hormones are responsible for the transport of sperm to the site of fertilisation and also transport of the zygote to the uterus. The oviducal contents provide an optimum environment in which fertilization and early embryonic development occurs. The volume of fluid secreted in the oviduct varies depending upon the stage of oestrous cycle. It is lowest during luteal phase, increases at the oestrus, reaches to the maximum one day after heat and then declines. The rate of secretion is governed by the

Table 5  
BIOMETRY OF FALLOPIAN TUBES IN BUFFALOES

Reference	Sane <i>et al</i> , 1964	Sane <i>et al</i> , 1965	Kaikini, 1974	Kodagali <i>et al</i> , 1971
Breed	Murrah	Jaffri	Berari (Nagpuri)	Surti
No. of observations	877	214	950	330
Length (cm.) Left	22.38 $\pm$ 0.12	24.49 $\pm$ 0.28	19.60 $\pm$ 2.97	19.80
Width (cm.) Left	0.2	0.2	0.2	0.27
Length (cm.) Right	22.56 $\pm$ 0.12	24.41 $\pm$ 0.27	19.54 $\pm$ 3.00	18.82
Width (cm.) Right	0.2	0.2	0.2	0.27

than those of the cow and are more coarser. The measurements reported by various authors are given in Table 5.

### Oviduct of Mare

The oviducts of mare are 20-30 cm. in length and 2-3 mm. in width, tortuous, wiry and hard. The bursa is very narrow, placed close to the ovulation fossa. Its uterine end joins the abrupt end of the corresponding horn. The oviduct opens on a muscular papilla in the uterus and therefore diseases of oviducts are possibly rare in the mare.

### Oviducts of Ewe and Goats

Oviducts are difficult to be distinguished from the uterus as there is no distinct demarcation. The tube is very tortuous close to the infundibulum. The surface area of bursa is 6-10 sq. cm. in the ewe. According to Puranik and Kaikini (1968) the length of right and left fallopian tubes were 17.56 and 19.02 cm. based on 197 observations.

### Oviduct of Sow

The oviducts of sow are about 15.30 cm. in length. The bursa is well developed and it largely encloses the ovary.

### Oviducts of bitch and cat

Oviducts of bitch and cat are smaller, about 5-7 cm. in length and surrounded by a large amount of fat. The oviduct passes first forward in the lateral part of the pouch and later runs backwards in the medial part. The dog is the only species in which oviduct encircles the bursa except for a slit which is ventro-medial. This slit increases in length during pro-oestrus from 1 to 3 mm. and a reddish coloured mass (rotliche mass) protrudes into the peritoneal cavity.

### Blood and nerve supply

The arterial blood supply to fallopian tubes is derived from utero-ovarian arteries. The veins are satellites of the arteries. The lymph vessels drain to lumbar lymph glands. The nerves are derived from renal and aortic plexuses and accompany arterial branches.

## UTERUS

### Uterus of Cow

It is a hollow elongated musculo-membranous sac consisting of two uterine cornua, corpus uteri and cervix.

Bovine uterus is 'Y' shaped. The shape, arrangement of cornua and relative proportion of component parts vary in different species. It is continuous anteriorly with fallopian tubes and posteriorly with vagina.

The uterus is situated in pelvic cavity and does not extend beyond the external angle of ilium. It is related to the rectum above, bladder below and intestines in front. It is covered by peritoneum and attached dorso-laterally to the pelvic cavity by broad ligaments through which the uterine blood supply and nerve supply is received. The blood vessels are many in number, thick walled and tortuous.

The cow uterus is bicornuate bipartite type (uterus bicornuatus bipartite). The cornua are well developed and lie almost parallel to each other. The lesser curvature is concave and directed downwards. Each cornua measures about 25 to 40 cm. in length and 1.5 to 5 cm. in width, thin and tapering towards the cranial end. They are joined by dorsal and ventral inter-cornual ligaments. The curvature of the uterine cornua resembles the curly horns of a ram. Each is directed forwards, outwards, downwards, backwards and upwards to form a spiral curve. The cornua are firm and freely movable. Tone of the uterus varies according to the physiological status.

Posteriorly the cornua open into body of uterus which is not very well defined in the bovine and measures about 2.5 to 3 cm.

## Endometrium

The mucosa consists of an epithelial lining a glandular layer and connective tissue. The thickness and vascularity varies according to the stage of oestrous cycle and gestation. In ruminants, the interior of the uterus presents mushroom like non-glandular projections called as caruncles. These are arranged in four rows. The inter-caruncular spaces contain many blood vessels and uterine glands. About 70 to 120 caruncles convex in shape, each measuring about 1.5 x 1.5 cm. and devoid of glands are present in the uterus. They may attain a diameter of about 10-12 x 3.75 x 2.5 cm. during pregnancy and appear spongy due to development of numerous crypts receiving the chorionic villi of the placenta.

## Uterine glands

The uterine glands are located throughout the endometrium except in the region of caruncles. They are branched tubular glands which are coiled at the ends. The number of the glands and their density depends upon species, breed, stage of oestrous cycle and parity. In the cornua, the uterine glands are plenty whereas they are very few in the mucosa near the cervix. It is considered that these glands provide nutrition to the zygote before implantation.

early diestrous stage, the endometrium increases in thickness, the surface epithelium becomes highly columnar and the uterine glands assume maximum development. During this stage, the uterine glands are actively secretory. In late diestrus phase the endometrium shrinks, the size of the glands diminish and there is very little secretory activity. These changes are noticed irrespective of fertilization of ovum. The cyclic changes in the endometrium such as shedding of the epithelium followed by regeneration hardly account for any extravasation of blood while as in the primates a large portion of the endometrium is usually shed resulting into extravasation of blood. During metestrous bleeding in the cow, the caruncles show capillary distention but no changes are seen in the epithelium. Destruction of the epithelium and capillary haemorrhage does occur inbetween caruncular spaces (Weber *et al*, 1948).

### Myometrium

The muscular layer of the uterus consists of the inner circular layer and the thin outer longitudinal layer. The connective tissue inbetween caruncular stalks contains nerves, blood and lymph vessels. During pregnancy there is hypertrophy and hyperplasia of the smooth muscle cells. The longitudinal fibres shorten the uterus and the circular fibres decrease the lumen on contraction which assists in the propulsion of the foetus during parturition.

### Serosa

The serous coat is the outermost coat and together with the vascular and the outer longitudinal muscle fibres holds the uterus to the dorso-lateral sides of the pelvis.

### Uterine Motility

The degree and the frequency of the uterine contractions vary in different stages of oestrous cycle. The uterus shows slow, feeble and irregular movements during diestrus. During oestrus, the contractions are rhythmic, peristaltic, wave like beginning at the apex of the uterus. The rate and amplitude increases but after ovulation the uterine muscles become quiescent. This quiescence is maintained throughout the period of gestation.

### Blood supply

The uterus is supplied with blood by middle uterine artery, utero-ovarian artery and posterior uterine artery, a branch of internal pudic. Blood vessels travel through broad ligaments. Veins are satellites of arteries, lack valves and form pampiniform plexus. Lymphatics pass to iliac lymph glands. Sympathetic nerves are derived from mesenteric, uterine and pelvic plexuses.

### Functions

The uterus has many important functions. During coitus the uterine contractions assist in the transport of sperm to the fallopian tubes. During early life the embryo is nourished by the uterine fluid. Formation of the placenta and progressive growth of foetus occurs in the uterus. The size, structure and position of uterus undergoes changes during oestrus cycle, gestation, parturition and involution. The uterine contractions play a major role in expulsion of the foetus during parturition.

### Uterus of buffalo

Uterus of buffalo resembles that of cow but is relatively larger and well defined. It is bluish grey in colour and striped with superficial veins. These

Table 6  
BIOMETRY OF UTERINE CORNUA IN BUFFALOES

Authors	Sane <i>et al.</i> , 1964	Sane <i>et al.</i> , 1965	Kaikini, 1974	Kodagali <i>et al.</i> , 1971
Breed No. of observations	Murrah 877	Jaffri 214	Berari 989	Surti 330
Left cornua Length (cm.)	38.75 ± 0.18	50.27 ± 0.35	31.20 ± 11.32	24.92
Width (cm.)	2.62 ± 0.01	2.73 ± 0.03	—	2.42
Right cornua Length (cm.)	39.13 ± 0.18	51.33 ± 0.03	31.55 ± 11.78	25.21
Width (cm.)	2.8 ± 0.02	2.73 ± 0.03	—	2.50

are not clearly seen in cows. The uterine cornua are more muscular and tortuous. The body of uterus is smaller and measures 1.38 cm. The broad ligaments are thinner and not muscular as in Egyptian buffaloes. The intercornual ligaments are more closely adherent to the cornua than in the cow. Kodagali *et al* (1971) reported the number of cotyledons to be 56 in each cornua. The measurements of uterine cornua in buffaloes are presented in Table 6.

apparent. The cornua terminate abruptly towards the ovarian end where the diameter may vary from 2.3 cm.

The uterus is located dorsally to the bladder. The anterior border of the body and cornua is usually situated in the form of an arc at the level of pelvic inlet.

The musculature of uterus consists of three layers viz. circular, longitudinal and oblique. The uterine glands are smaller. The caruncles are not present on the mucosa which is lined by high columnar epithelium and thrown into prominent longitudinal folds.



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The serous coat is the outermost coat and together with the vascular and the outer longitudinal muscle fibres holds the uterus to the dorso-lateral sides of the pelvis.

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The degree and the frequency of the uterine contractions vary in different stages of oestrous cycle. The uterus shows slow, feeble and irregular movements during diestrus. During oestrus, the contractions are rhythmic, peristaltic, wave like beginning at the apex of the uterus. The rate and amplitude increases but after ovulation the uterine muscles become quiescent. This quiescence is maintained throughout the period of gestation.

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Width (cm.)	2.8 $\pm$ 0.02	2.73 $\pm$ 0.03	—	2.50

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The musculature of uterus consists of three layers viz. circular, longitudinal and oblique. The uterine glands are smaller. The caruncles are not present on the mucosa which is lined by high columnar epithelium and thrown into prominent longitudinal folds.

as 17.04 and 1.58 cm. respectively. The corresponding figures for left cornua were 17.00 and 1.55 cm.

### **Uterus of Sow**

The uterine cornua of sow are about 120-150 cm. in length, tortuous and resemble thick walled intestines. The body is very short about 5 cm. in length. Broad ligaments usually contain large amount of fat. Endometrium has usually prominent longitudinal folds which fade gradually towards cervix.

The uterine glands provide nutrition to the embryo during the entire gestation period.

### **Uterus of Bitch and Cat**

The uterine cornua are straight about 12 to 15 cm. in length and diverge in form "V" from the body towards the kidney. The cornua present ampullae which are separated by constrictions during pregnancy. The foetuses are located in these ampullae. The body is about 2.5 cm. long. The uterus lies entirely in the abdominal cavity suspended from the sublumbar region.

## **CERVIX**

### **Cervix of the cow**

The cervix is the constricted compact portion of uterus continuous anteriorly with the corpus uteri. It projects into the vagina like a knob at the centre of which is the opening called os uteri externum. Anteriorly the cervix opens into the body by os uteri internum. The distance between these two is the cervical canal. Length and width of cervix varies from 7.5 to 10 cm. and 2.0 to 6.0 cm. respectively. The cervical canal is a narrow curved passage.

The mucous membrane of cervix presents 3-5 transverse folds, the last one of which enters into the formation of os uteri externum. In pluriparous cows, the last cervical fold usually protrudes out. There is progressive physiological hypertrophy during various stages of gestation in the cow. This is markedly observed in pluripara.

The cervix consists of the mucosa, muscular layer and a serous coat. Mucosa is arranged in the form of conspicuous folds from which secondary folds arise giving rise to a fern leaf like appearance. The mucosa is non-glandular but the mucus secreting cells are plenty. The primary and secondary folds of mucosa thus have a very large secretory surface. During oestrus, greatest secretory activity follows and the mucus is less viscous.

The muscularis consists of inner circular layer which forms the main substance of annular folds. It is rich in white fibrous and elastic tissue and smooth muscle cells.

### **Functions of the cervix**

The secretions from the uterus and cervix during oestrus are voided through the cervix. It acts as a passage, for storage and transport of sperms during oestrus. It acts as a barrier against ascending types of infections. Special glands "ovula nabothi" present in the cervical mucosa secrete a thick mucus which acts as a seal during gestation. At the onset of parturition, the cervical mucous seal liquefies followed by relaxation of cervix.

### **Cervix of the buffalo**

The cervix is narrower and shorter as compared to cow (Table 7). Physiological hypertrophy of cervix as it occurs in cows is not observed in buffaloes.

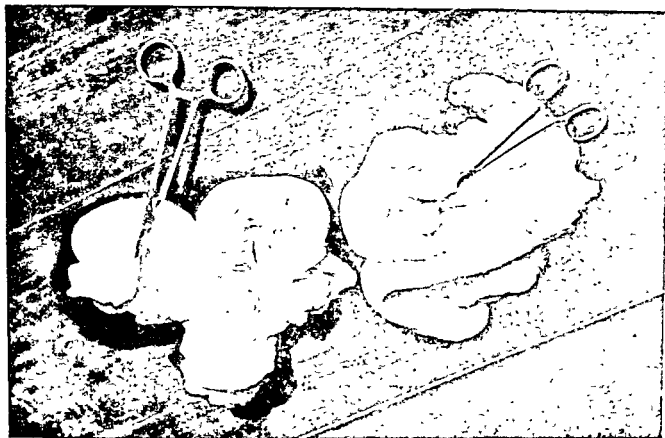


Fig. 11. Uterus and ovary of buffalo.



Table 7  
BIOMETRY OF BUFFALO CERVIX

Authors	Sane <i>et al.</i> , 1964	Sane <i>et al.</i> , 1965	Kaikini, 1974	Kodagali <i>et al.</i> , 1971
Breed	Murrah	Jaffri	Berari (Nagpuri)	Surti
No. of observations	877	214	1015	330
<b>Cervix</b>				
Length (cm.)	7.82	8.09 $\pm$ 0.13	6.85 $\pm$ 2.68	5.54
Width (cm.)	2.79	2.95 $\pm$ 0.04	—	2.65
Thickness (cm.)	—	—	1.68 $\pm$ 0.60	2.25
No. of annular folds	2-5 (av. 3)	2-5 (av. 3)	3.43 $\pm$ 0.81	3.45

### Cervix of the Mare

The cervix is about 5-8 cm. in length and 3.75 cm. in diameter. It is relatively shorter than that of the cow. The portio vaginalis projects in the vagina for about 2.5 to 5 cm and cervical opening is usually found relaxed except during pregnancy. The cervical mucosa is thrown into longitudinal folds instead of transverse annular folds as in the cow.

### Cervix of Ewe and Goat

The cervix in the ewe and goats resembles that of the cow. The cervical canal is about 3.75 cm in length Purnik and Kaikini (1968) reported the mean length of cervix as 3.26 cm based on 210 observations.

### Cervix of Sow

Cervix is poorly defined. It presents 2.3 cm thick longitudinal cervical folds. Portio vaginalis is absent.

### Cervix of Bitch and Cat

Cervix is short and projects in the vagina in the form of a small thick prominence.

## VAGINA

### Vagina of the Cow

The vagina is a musculo-membranous organ. It extends from cervix backwards and is related to sacrum and rectum above, bladder, urethra and pelvic floor below. Laterally it is related to the sides of pelvis. It serves as a copulatory organ and passage for expulsion of foetus during parturition.

The length and diameter of vagina varies from 17 to 25 cm and 10-15 cm respectively. Anterior two-thirds is covered by peritoneum which is attached to the roof of pelvis by broad ligament whereas the remaining retroperitoneal part is attached to neighbouring structures by loose connective tissue.

with sensory nerves. The corium consists of vascular and dense connective tissue. The muscular coat has inner circular and outer longitudinal layer. This layer is also supplied with blood vessels, nerve bundles, small groups of nerve cells and loose connective tissue. In the cow, the anterior and posterior sphincters are present whereas, only posterior sphincter is present in other farm mammals. The cyclical changes can be studied from the vaginal smears to a certain extent (Deopurkar, 1974).

The presence of lymphoid band anterior to urinary meatus prevents ascending infection to a considerable extent (Bane, 1965).

The canals of Gartner commencing in the broad ligaments extend between the mucous and the muscular layer. They open in front and by the sides of urinary meatus. These are absent in the mare, ewe, goat, bitch and cat. They are the remnants of Wolffian duct and their function is not clearly known.

#### Blood supply

Blood supply to vagina is provided by internal pudic artery. Numerous veins join the internal pudic vein and lymphatics are drained by internal iliac lymph glands. Sympathetic nerves are derived from pelvic plexus.

#### Vagina of the buffalo

Vagina of the buffalo resembles that of the cow and is less contractile and flaccid. The length, width and thickness of vagina in Murrah buffaloes is 21.36, 6.44 and 0.46 cm. respectively (Sane *et al*, 1964).

#### Vagina of the Mare

It is about 20-25 cm. in length and 10-12 cm. in diameter. The mucus membrane is thrown into longitudinal folds. Musculature of vaginal wall is

thicker and the fornix vaginae is very distinct.

#### Vagina of the ewe and goat

Vagina of ewe and goat is about 8-14 cm. in length. Its ventral part contains many lymph follicles.

#### Vagina of the sow

It is 10-15 cm. in length. Its diameter is smaller but musculature is thicker. It is thrown into longitudinal folds. Fornix is not distinct.

#### Vagina of the bitch and cat

The vagina is long and the fornix is not distinct. Mucus membrane is thrown into longitudinal and transverse folds. It is divided in two sections (i) the cranial part extending from the cervix to the point of urinary meatus and (ii) a shorter caudal part which is common to the genital and urinary tract. The first part constitutes the vagina and the second the vestibule. The retroperitoneal part of the vagina is largely covered with considerable amount of fat. When the vaginal speculum is introduced, it will be seen that due to the extensive mucosal folds, the passage becomes obliterated due to which the cervix is not clearly seen. The collapse of the walls at the end of the speculum may give a false impression of the external os. The vagina has a considerable capacity to extend. Hymen is rarely present.

The vestibule is continuation of the vagina upto the pudendal or vulval cleft. It has a pronounced inclination caudo-ventrally. Vestibular mucous membrane is smooth and the deeper layers are inelastic. Urethra enters in the proximal part of vestibule and a fossa is present on either side. Vestibular glands are rarely present.

## VULVA

### Vulva of the cow

It is the posterior most segment of the genital tract situated 5 to 7 cm. below the anus. It originates from the ectoderm and unites with the vagina at the vulvo-vaginal border-hymen. The vulva is related to anus dorsally, to ischial arch ventrally, sacro sciatic ligaments and semimembranosus muscles laterally. There is no distinct line of demarcation between vulva and vagina. Vestibular length in cow varies from 10-12 cm.

The outer opening is in the form of a slit bound by two labia which meet at upper and lower commissures. The size and appearance of vulva varies according to the age and reproductive status of the animal. Labia are large, soft, thick and normally wrinkled but become thick and turgid when the cow is in oestrus. Upper commissure is acute and separated from anus by a short distance. The lower commissure is elongated and bears a tuft of hairs and is located 5 cm. below and behind the ischial arch.

### Hymen

It is a transverse membranous partition situated in front of meatus urina-rius between vulva and vagina. It usually disappears during early life or by pubertal age. Due to developmental abnormality the partition between hind-gut and proctoderm persists in the form of hymen — a membranous band connecting the roof and floor of vaginal passage. This may lead to accumulation of uterovaginal secretions.

### Histology

The labia are provided with several circular or sphincter like muscles which keep them in close apposition. They consist of anterior and posterior constrictor muscles. Attachments of vulva to pelvis and other related structures afford a fixed base. During parturition, this fixation enables the uterus to dilate cervix, vagina and vulva.

are elongated in form and not conical as in the cow. They measure 12.48 cm. in length and 12.46 cm. in width in Murrah buffaloes (Sane *et al*, 1964). Tuft of hair at the ventral commissure is absent. The clitoris in the buffalo is not conspicuous as in cows.

### **Vulva of the Mare**

The length of vulva is about 10 to 12.0 cm. The labia are shorter, thinner and non-wrinkled. Upper commissure is acute and the lower one rounded. The constrictor muscles and the erector bulb are highly developed. 8 to 10 ducts of each Bartholin's gland open on the dorso-lateral wall of the vulva. Clitoris is large, flexuous and prominent, 2.5 to 5.0 cm. in length and 2 to 2.5 cm. in width. Meatus urinarius is capacious and can admit 2-3 fingers.

### **Vulva of the ewe and goat**

The vulva resembles that of the cow but is shorter and 2.5 to 3.0 cm. in length. The sub-urethral diverticulum is shallow. Clitoris is not conspicuous. The labia are thick and the ventral commissure projects downwards. Vestibular glands are frequently absent.

### **Vulva of the sow**

The vulva is about 8.75 cm. in length. The labia are very thick and wrinkled. The ventral commissure is more acute and pointed and the dorsal commissure is rounded. Clitoris is prominent, sinuous and pointed. Canals of Gartner are flexuous and open in a depression situated between meatus urinarius and fossa clitoridis. Sub-urethral diverticulum is more or less similar as that in the cow.

### **Vulva of the bitch and cat**

The vulval opening is triangular. The labia are thick and perineum is very

wide as compared to cow, buffalo and mare. Urethra opens on a urethral tubercle and there is no valve for meatus urinarius as in other animals. Glands of Bartholin are absent in dog but present in the cat. Clitoris is broad, flat, 3.75 cm. in length and pointed. It is infiltrated with fat. In cats a visceral bone may be present in clitoris.

The large and irregular fossa of the clitoris is situated at the distal extremity which is relatively inconspicuous as it is roofed by a fold of mucosa. Vestibular glands are rarely observed. Circularly arranged muscles at the external surface of the vestibule cause contractions and retain the penis during copulation prolonging the tie which is characteristic of the species.

## **MAMMARY GLAND**

Although these are cutaneous glands, they are considered accessory glands of the reproductive system on account of their close physiological association. These secrete milk for nourishment of young one. The udder is attached by suspensory ligaments to the posterior and lower part of abdomen. The development of glandular structure is primarily dependant on the genetic make up followed by hormonal influence exerted at sexual maturity, during gestation and lactation. The shape of udder is hemispherical and its position is inguinal. It consists of four quarters, each having a teat about 6.25 to 8.25 cm. in length. The length of teat varies according to age and number of lactation. Teat has a single opening connected with the corresponding lactiferous sinus by a teat canal. The sphincter at the tip of teat is well developed. Super numerary teats are seen in some cows.



## Histology

Microscopically, the udder tissue consists of four layers — the skin, outer fibrous, intermediate fibrous, inner fibrous and the mucosa. The circular and longitudinal muscle fibres form a well defined sphincter about 1.25 cm. in length at the apex of teat. Parenchyma is yellowish and pink. It consists of a racemose type of secretory tubules with alveoli, ducts, capillary nets and delicate connective tissue with fine net work of elastic tissue. The gland is of apocrine type and therefore the lining epithelium varies according to the secretory status.

## Blood Supply

The blood supply is derived from subcutaneous mammary and perineal arteries—the branches of external pubic. The veins accompany arteries and they form plexus on either side of the base of glands. Lymphatics are numerous and lead to supramammary and lumbar lymph glands. Nerve supply is derived from inguinal and posterior mesenteric plexus of sympathetic system.

form a lobule and many lobules form a lobe. These lobes and lobules are connected by a series of ducts. The diameter of alveoli varies from 0.1260 to 0.2340 mm. with an average of 0.18 mm. In majority of buffaloes corpora amy-lacea  $72\ \mu$  in size are found as spheroid concretions. The histological picture varies according to the stage of lactation (Mehendale, 1967).

## Mammary glands of the Mare

The mammary glands of the mare are hemispherical and two in number. The teats are smaller compared to the cow and have 2-3 openings. Lactiferous sinuses are two in number and the sphincter is not well developed.

## Mammary glands of the ewe and goats

The mammary glands are two in number. The glands, the lactiferous sinuses and the teats are relatively larger. Rudimentary teats may be observed in some ewes and goats.

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# Chapter 3

## Accidents During and Incidental to Pregnancy

### ABORTION

The origin of the word 'abortion' has a latin root—Ab=abnormal and oriri=to be born. Abortion is the expulsion of living or dead foetus of recognisable size before term. Generally the aborted foetuses are non-viable. The condition in which fertilized eggs and embryos are lost before the recognizable size is reached, is known as early embryonic death.

### I. NON-INFECTIOUS CAUSES

#### A. Abortions caused by trauma or accidental abortions (Physical causes)

Abortions in cattle may be caused by trauma or accidental injuries such as, fall from a high level, prolonged struggling, exertions subsequent to fighting, chasing by a dog, exposure to extreme climatic conditions, vaccination of a pregnant animal, passing through a narrow gate, jolting during transport, irregular feeding and jumping over a ditch. *Goadng or butting on the right flank of a pregnant cow by other animals* may also lead to abortion. Careless treatment for infertility such as uterine irrigations with irritant antiseptics, douching, introduction of a pipette/catheter in a pregnant animal, dilation of cervix, inadvertent enuclea-

is little authentic proof to substantiate this view. As far as is known, no chemical drug except oestrogens and ergots have abortifacient actions. However, following drugs have been incriminated as causal factors for abortion:

(i) Chlorinated naphthalene (Hansel and Mc Entee, 1955), potassium nitrate (Nitrate or low land and/or marshland abortions—Simmon *et al*, 1958), lead poisoning due to licking of oil paints.

(ii) Arsenic poisoning due to dips, due to dihydrocarbons and organophosphorus compounds.

(iii) Locoweeds (*Astragalus lentiginosus*, *A. pubentesimus oxytropis sericea* broom weed of *Gutierrezia* species.—Dollahite, 1962), pine needles (*Pinus ponderosa*), Tucker (1961), Bracken (1968), Faulkner (1968).

(iv) Purgatives such as castor oil, calomel, aloes, etc.

(v) Pituitary and lentin.

(vi) Sodium iodide, selenium (Beath *et al*, 1953).

(vii) Ergot (Mantle and Gunner, 1965).

(viii) Anthelmintics such as carbon tetra-chloride, phenothiazine.

(ix) Quinine, pyrophosphorus and digitalis.

(x) Toxic plants.

(xi) Sweet clover hay or musty or mouldy silage (Bracken, 1968).

### C. Psychological factors

Abortions due to psychological factors in cattle are rare. Lagerlof (1969) and Hofflund (1969) reported that roaring noise of the aeroplanes and helicopters passing close to the stables and byres and bombing tests in Switzerland were responsible for abortions in cows, mares and sheep. Bane (1969) considers lightning flash to be more dangerous than a thunder storm and may possibly be due to hormonal upset.

### D. Hormonal factors

Oestrogens, diethyl stilboestrol, E.C.P. or Oestrogen pellets administration may lead to abortions in various stages of pregnancy. Rankin (1963) reported abortions in two herds in Ireland due to excessive oestrogens in pasture. The poultry litter from birds consuming oestrogens from feed when fed to cattle causes abortion (Griel *et al*, 1969). The gluco-corticoids may cause abortion in cattle, since there is close relationship between adrenal cortex and maintenance of pregnancy (Van-Rensberg, 1965). Hyperplasia of the adrenal gland is reported to cause abortion in sheep (Liggins, 1968).

Progesterone deficiency may cause early abortion between 45 to 185 days, 83% occurring before hundred days of gestation (Morrow, 1968). This probably may be due to unstable endocrine constitution. Woelffer (1953) could prevent abortion in a habitual cow by injection of 500 mg. of repositol progesterone every ten days from 4-5 months to three weeks before term.

Pituitrin or oxytocin injections during gestation period do not cause abortion in animals (Roberts, 1971). However, instances are on record in pregnant buffaloes that abortions have occurred subsequent to administration of pituitrin for let down of milk (Hukeri, 1977).

### E. Nutritional deficiencies

Multiple nutritional deficiencies may occasionally produce abortions. The deficiencies may be multiple or specific. In such cases, emaciation is a prominent symptom in cattle with high incidence of abortions, birth of dead, weak or premature calves. Similar conditions existed in Sweden during 1910 but abortions were rarely observed (Lagerlof, 1969). Under famine conditions in India,

abortions are rare in cattle. However, during extreme summer conditions in Kaira district of India, there is scarcity of green fodder which causes Vitamin A deficiency for a long period in consequence of which abortions occur in buffaloes (Chothani, 1972).

The other specific deficiency includes Vitamin A which adversely affects reproduction (Thomas, 1968). Vitamin A deficiency causes eye defects and keratinisation of vaginal epithelium and degeneration of placenta which may lead to abortion. Dystokia may occur followed by infection and retention of placenta.

Lack of calcium, selenium and iodine (Alcroft *et al.*, 1954), selenium (Mace *et al.*, 1963), cobalt, iron and copper deficiencies may also cause abortion.

reported abortions in 3 cows bred to a particular bull whose blood was used for transfusion. They concluded that circulating maternal erythrocyte antibodies were responsible for abortions. However, iso-immunisation of pregnancy has not yet been proved to be the cause of abortion.

Abortions may also occur subsequent to torsion of uterus, strangulation of uterus by a lipoma and lack of caruncular area in the uterus. All these may lead to death of foetus and abortion. Twinning, allergy and anaphylactic reactions may also cause abortion in cattle (Roberts, 1971).

Severe burns, possibly on account of histamine like substances may result in abortion. Madhavan (1969) reported abortions in buffaloes where skin area was burnt upto 25%.

Infectious abortions are of two types, viz.: (A) contagious (epizootic), and (B) Non-contagious (enzootic).

### A. Contagious abortion

Contagious abortions are associated with following diseases: (a) *Bacterial diseases*: (1) Brucellosis. (2) Leptospirosis. (3) Vibriosis. (4) Listeriosis. (5) *Salmonella abortus*. (6) *Pasteurella tulerensis* in sheep.

### B. Viral diseases

(1) Infectious bovine rhinotracheitis and infectious pustular vulvo-vaginitis (IBR-IPV). (2) Epizootic bovine abortion (EBA). (3) Rift valley fever (RVF). (4) Wessel Bron (WB-virus). (5) Bovine virus diarrhoea and mucosal disease (BVD-MD). (6) Myxo virus para-influenza III. (7) Pseudo rabies (Aujeszky's disease). (8) Picorna virus (SMEDMI virus). (9) Equine arteritis virus. (10) Epizootic abortion of ewes (EAE). (11) Blue tongue virus. (12) Coxiella or Rickettsia burneti or Q-fever virus.

### C. Mycotic (Fungal) diseases

(1) *Aspergillus fumigatus*. (2) *Abisidia ramosa*. (3) *Mucor pusillus*. (4) *Rhizopus arrhizus*. (5) *Monosporium apiospermum*. (6) *Cephalosporium*. (7) *Humisola*. (8) *Candida tropicalis*. (9) *Penicillium*. (10) *Syncephalastrum racimosum*.

### D. Protozoal diseases

(1) *Trichomonas foetus*. (2) Toxoplasmosis. (3) *Trypanosoma equiperdum*. (4) Pyroplasmosis.

### E. Miscellaneous diseases

(1) Tuberculosis. (2) *Corynebacterium pyogenes*. (3) *Pseudomonas*

aeruginosa. (4) *Aeromonas hydrophila*. (5) Streptococci. (6) Staphylococci. (7) Coliform bacilli. (8) *Erysipelothrix insidiosa*. (9) *Haemophilus*. (10) *Spherothorus necrophorus*.

The organisms included in the above list cause abortion in domestic animals at various stages of pregnancy and their symptomatology, diagnosis, treatment and prevention of commonly occurring ones has been dealt with in details in chapter on Infectious infertility.

### Symptoms of abortion

Early abortions usually go unnoticed. However, in stabled animals, the aborted embryo with slipped membranes can be noticed in the rear of the animal during routine check up. When the animals are kept loose in the paddock, these membranes are likely to be trampled and soiled with dung and urine.

In early abortions, the animals return to service very early.

In the second trimester of pregnancy, animals may abort without any apparent symptoms. In some animals, mild straining with elevated tail, peculiar bellowing, relaxation of diaphragma pelvis and vulva, liquefaction of cervical seal and appearance of sero-sanguinous discharge at vulva are noticed.

Symptoms indicative of threatened abortion are increased uterine contractions, closely crowded cotyledons and rigid uterine walls. The findings should be communicated to the owner and re-examination of the animal is advised.

### Extra uterine pregnancy (Ectopic pregnancy)

Normally a foetus develops in the endometrium but rarely the foetus may be found located at various situations such as ovary, fallopian tube, mesentery,

omentum, interstitial tissues, uterus and vagina. Such pregnancies are known as extra uterine or ectopic pregnancies and may be classified as true and false or primary and secondary.

### **True extra uterine pregnancy**

The term primary or true extra uterine pregnancy is applied to a fertilized ovum, embryo or foetus which receives nutrition from organs or tissues other than endometrium and which has attained a certain degree of embryological development. The occurrence of tubal and ovarian pregnancy is very common in human beings, however in animals, this has not been reported so far. This might be due to variation in placentation of primates and animals. In the former, the developing zygote erodes the mucosa and establishes there, whereas in the latter, the trophoblastic villi attach themselves in the maternal endometrial crypts. Obviously, the peritoneal surface is not a favourable site for placentation, but the development of a full term foetus which later mummified, has been recorded in the dog by Schlotthauer and Wakim (1955).

usually during the late stages of pregnancy. In many cases, a sterile foetus is released into the abdominal cavity without any visible external symptoms. The dead foetus and its envelopes become walled off as a sterile foreign body in the ventral portion of abdominal cavity and may not be detected until after slaughter (Spanabel, 1957). In human, released extra uterine foetuses become harder and encapsulated with calcium deposits known as "Lithopedions". This condition has to be differentiated from mummification, tumours, fat necrosis, rotated or compound bicornual pregnancy in mare. The correct diagnosis can be arrived at by critical manual examination in large animals and abdominal palpation, radiograph or exploratory laparotomy in small animals. Fatal peritonitis and shock is a sequelae if an emphysematous foetus escapes into the peritoneal cavity.

### **Antepartum paraplegia of pregnancy (Paraplegia of pregnancy or paresis)**

This condition is usually observed in advanced pregnancy when the foetus, uterus and udder are undergoing rapid

(2) KETOSIS (PREGNANCY TOXAEMIA IN EWES, PREGNANCY DISEASE, TWIN LAMB DISEASE OF SHEEP)

Advanced pregnant ewes often suffer from debility and paraplegia due to lack of carbohydrates in diet. The general behaviour is sluggish and appetite is poor. The foetus dies and the ewe succumbs. The affected ewes are usually thin but the condition may also be observed in healthy ewes. Confinement to pens may precipitate the condition. The ewes become dull, anorexic, paretic and are disinclined to rise. Nervous symptoms such as, moving in circles, pressing the head against hard object, raising the head in the upward direction or to one side, may become obvious. Urine and blood on microscopic examination reveal large quantities of ketone bodies. Unless prompt treatment is instituted, the symptoms tend to be progressive or unless abortion occurs, there is a high rate of mortality in ewes. On post mortem examination the liver shows fatty changes.

Treatment comprises of intravenous injection of 200 ml. of 40% glucose, together with injections of 100-200 mgm. of cortisone or 50-100 I.U. of ACTH. Efosin, a specific antispasmodic for cervix may be of some use (Narsimhan and Thangaraj, 1969). Caesarian may be necessary in certain ewes. Additional energy intake and sufficient exercise are recommended for other pregnant ewes.

(3) GRASS TETANY

This condition is usually due to hypocalcaemia or hypomagnesaemia. According to Kronfield and Simensen (1970), the high potassium or high level of Vitamin A or transaconitrate content in grass is the real cause of depletion of calcium and magnesium from the body. This drain on calcium

and magnesium becomes more marked during advanced pregnancy and heavy lactation. It is better to withdraw the cattle from lush pasture for sometime. Calcium and magnesium therapy has proved efficacious in paretic cases with obvious nervous symptoms.

(4) MILK FEVER (PARTURIENT PARESIS)

A high yielding dairy cow often suffers from preparturient milk fever or parturient paresis just prior to parturition; mostly during first stage during which let down of milk is triggered by the oxytocin release. Such cases show low blood calcium levels and respond favourably to intravenous calcium therapy.

A similar condition in dogs and cats is known as puerperal tetany or eclampsia and is characterised by hypocalcaemia, restlessness, whining, staggering, and clonic spasms. This is observed within first two weeks postpartum.

(5) DROPSY OF FOETAL MEMBRANES AND FOETUS

The cow may be unable to rise due to hydrops amnii or hydrops uteri and consequent increase in the abdominal weight.

(6) JOINT AND TENDON INJURIES

Dislocation of hip joint is common in weak, high pregnant cows and buffaloes stabled on slippery or wet floors. Accidental rupture of prepubic tendon in mare or rupture of gastrocnemius tendon secondary to Zenkar's muscle degeneration in cows, so also fractures of pelvis in female may lead to inability to rise and paraplegia. Mounting by a bull or cow on pregnant animal, may cause fracture of sacral and coccygeal vertebrae resulting in paralysis of tail.





FIG 14a Dropsy of foetal membranes  
in a buffalo





Fig. 14c. Antepartum cervico-vaginal prolapse and prolapse of rectum in a cow.

←



Fig. 14d. Uterine hernia (hysterocoele) in a cow.

←



Fig. 14e. Haematinic mummification in a cow.

**(7) SEPTIC OR INFECTIVE PROCESSES**

Septic metritis, rupture of uterus, severe peritonitis, traumatic gastritis, torsion of uterus, rupture of uterus, pyelonephritis may be associated with paresis in advanced pregnant animals. Similarly, rupture of large uterine vessels, toxic indigestion, diseases of central nervous system viz. rabies and listeriosis, Johne's disease, severe mange, lymphocytoma of brain and spinal cord, anthrax, black quarter, leptospirosis, anaphismosis, severe foot lesions may contribute to weakness and paralysis particularly of hind limbs. The result is that the animal is unable to rise, remains in recumbency for a longer period and suffers from bed sores. These animals may improve with good nursing in a loose box with sufficient bedding of sand or straw and avoiding stone or concrete floors. Slings may prove useful in mares but cows and buffaloes are not comfortable in slings.

ures adopted. This haemorrhage may not be visible outside but large blood clots sufficient enough to cause death of dam may be observed around vagina and broad ligaments. Hence it is inadvisable to transport high pregnant mares or subject them to violent exercise.

**2 RUPTURE OF PREGNANT UTERUS**

This may occur spontaneously or subsequent to dystokia, emphysema of foetus, chronic peritonitis, torsion of uterus, dropsy of foetal membranes and foetus and violence during advanced pregnancy.

**3 STRANGULATION OF LARGE INTESTINE BY PREGNANT UTERUS**

Gravid uterus in a mare may displace the large colon causing digestive distress (Williams 1943).

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# Chapter 4

## PARTURITION

Normal parturition (eutokia, labour or natural birth) envisages various physiological processes involved in expulsion of normal viable foetus from the uterus through the maternal passage by natural forces alone at a stage when the young one is capable of independent existence (Roberts, 1971).

The act of parturition is termed as calving in cow, buffalo, camel and elephant, foaling in mare, lambing in ewe, kidding in goat, farrowing in sow, pupping or whelping in bitch, and kittening in cat. This act though physiological is accompanied by pain (*Dolores ad partum*), general disturbances, uneasiness and violent efforts in the dam, termed as 'labour pains'.

end. No two parturitions are similar within the individuals or species. Wide variations are encountered regarding the intensity, duration and frequency of labour pains and positions of the foetus.

### Factors initiating parturition

The immediate cause of parturition is not still very clear. Several factors such as physical, neural, biochemical and hormonal are involved in the process.

### A PHYSICAL FACTORS

- (i) *Progressive increase in the irritability of uterus*

(iii) *Infarcts in placenta*

At full term, infarcts are noticed in the placenta due to the distention and consequent pressure on the arteries. The blood supply is diminished and placenta becomes senile. The nutrition of the foetus is interfered with, it becomes anoxic, gasps and the respiratory centre is stimulated. The metabolic products of the foetus cause stimulation of the uterine centre and contractions are initiated (Eden, 1897 and Williams, 1930).

(iv) *Fatty degeneration in the placenta*

During the last stages of pregnancy, fatty degeneration of decidua (outer layer of placenta) occurs resulting into the separation of foetus and decidua. Foetus then is virtually converted into a foreign body. The uterus responds accordingly and the foetus is expelled out (Simpson, 1871).

## B. NEURAL FACTORS

(a) The spinal cord transection in dogs by Holtz and Frensborg (1874), Rein (1880) and Bard (1912) have clearly shown that parturition can occur independent of central nervous connections. Delivery can occur in women having spinal injuries with total destruction of spinal cord. Even the totally denervated human uterus can expel the foetus. There is no evidence to substantiate the functional relationship of the intrinsic innervation of the uterus

to its activity during labour (Raynold, 1949).

## C. BIOCHEMICAL FACTORS

(i) *Carbon dioxide tension*

Accumulation of  $\text{CO}_2$  in blood occurs due to the metabolic activities of the foetus which sets up uterine contractions. This can also be induced experimentally (Keiffers, 1896).

(ii) *Exciting Substances*

At full term the foetus transmits certain substances to the maternal circulation due to insufficient nutrition which in turn are believed to initiate parturition (Spiegelberg, 1891).

(iii) *Antigen*

According to Von der Heide (1911) an excessive quantity of foetal antigen enters the maternal blood stream towards the end of pregnancy. This interacts with the existing liberated substances by blood antigens and initiates labour. The exact nature of the antigen is not clearly understood.

## D. HORMONAL FACTORS

In this complex process, the known and unknown hormones from pituitary, ovary, adrenal, placenta, foetus and the uterus and probably prostaglandins act in a coordinated manner.

The levels of oxytocin and oestrogen optimum for normal parturition are shown in the following scheme (Lagerlof, 1965).

	Oestrogen	Oxytocin	
Normal contractions	+++	+++	} Optimum level
Weak ..	+	+++	
.. ..	+++	+	
			Subnormal level

At full term the rise in oestrogen level sensitizes the uterus to the action of oxytocin from pituitary. The oxytocin level in the blood is very low during the first stage of labour, increases during the second stage and thereafter it falls. Progesterone level drops due to the gradual degeneration of pregnancy corpus luteum. The uterine activity is largely dependant upon the hormonal balance between oestrogenic hormones and oxytocin on one hand and progesterone and like substances on the other. According to Csape (1950) the increase in oestrogen level causes an increase in actomycin — a contractile protein of uterine musculature. In addition to this, relaxin causes relaxation of pelvic ligaments and related structures. According to Hisaw and Zarrow (1950) it is produced in uterus under the influence of progesterone from corpus luteum. The role of relaxin is not yet fully understood. Whether the parturition is precipitated by the alteration of hormonal balance alone or depends upon several factors, is not yet clearly understood.

### Integrated mechanism

in turn stimulate uterine contractibility. Simultaneously, major blood flow is diverted towards the udder. Foetal expulsion is brought about by the relaxation of cervix, vagina and pelvic ligaments due to the action of relaxin and further enhanced by contractions of abdominal and uterine muscles during labour. Recently it has been proved that the levels of foetal plasma corticoids increase as the time of parturition approaches (McDonald, 1975).

### Onset of Parturition

It is difficult to predict the exact day and time of parturition. However, Jadhav (1975) reported that it is possible to predict the exact day and time of parturition in Gir cows by studying the ante-partum blood serum cholesterol content. It was 231.83 mg. % eight weeks prior to parturition and decreased at the rate of 10.82 mg. % per week till it touched 134.78 mg. % at parturition. The cholesterol level rose rapidly by 87.27 mg. % in the first week and 14.69 mg. % later upto eight weeks post-

over the metacarpal bones which forms a wedge to dilate the vagina. Once the head has cleared the vulva, the dam takes a little pause to allow the body tissues to get accustomed to this dilatation. By this time the chest has appeared in the pelvic cavity. The hips usually follow through the birth canal fairly rapidly and the further process is facilitated due to the weight from hanging parts of the foetal body. The hind limbs may remain in the passage until the foetus or the dam moves (Fig. 15 A, B, C, D, E, F, G).

During the second stage of labour the uterus contracts four to eight times, every ten minutes, each lasting for 80 to 100 seconds (VanderKaay cited by Roberts, 1971). Majority of the animals lie down as soon as straining commences, however calf and foal may be born in the standing position. The cow, bitch and ewe may recline resting on the sternum. The body is inclined to one side with forelimbs under the chest and hind limb under the abdomen (Fig. 16). The mare and sow usually lie flat with legs extended.

The intrauterine pressure of contractions according to Debruin cited by

Roberts (1971) was 66 mm. Hg. between the uterine contractions and 99 mm. Hg. during uterine contractions in the second stage. It reaches about 170 mm. Hg. on the commencement of straining. Thus the total pressure at the pelvic inlet is about 150 to 170 lbs. or about what one man can apply on traction of foetus.

The time required for this stage in cow and buffalo vary from half to three or four hours with an average of 0.5 to 1.00 hour in pluripara (Table 8 & 9). Primipara may take three hours or more. In mare, this stage is completed within 10 to 30 minutes, 30 minutes to two hours in ewe and goat and one to six hours in the sow.

The first pup in the bitch may take an hour and the time required for expulsion of subsequent pups is variable 2 to 6 hours depending upon the number of foetuses. If the foetus is delivered with dam in standing position, the umbilical cord ruptures by gravitational force whereas in the recumbent position the cord ruptures when the dam tries to rise up due to overstretching or due to foetal movements. On rupture, the

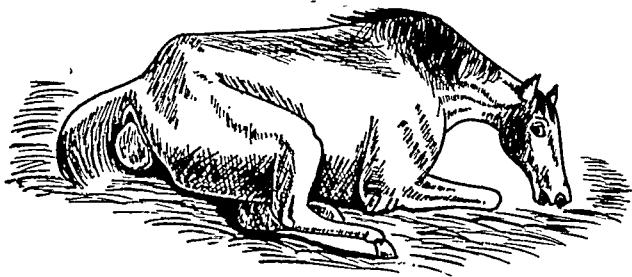


Fig. 16: Parturition in mare. Note the sternum recumbency.



particularly observed in primipara. The oedema which is physiological may be extensive on the abdomen and perineum. At times it is so extensive in young animals as to interfere with circulation and cause difficulty in gait. The skin covering the glands become red as seen chiefly in mares. Kadu and Kaikini (1975) recorded intense, medium and normal type of udder enlargements during the last trimester of pregnancy in Sahiwal cows in 10.1, 62.2 and 24.7% cases respectively. Serous fluid oozes out on pressure from the teats varying in consistency from watery to viscid colostrum. The milk cisterns get distended with colostrum which is a yellow, turbid, opaque and honey like cellular secretion.

The vulval lips become resilient and oedematous, two to six times their normal size. They become soft, flabby and parted. The mucus membrane becomes hyperaemic and covered with viscid mucus which later on becomes copious and it hangs in form of strings in the cow. Under the hormonal influence, the cervical seal gets liquified, cervix becomes softer and the os uteri is partially opened. The sacrosciatic ligaments appear sunken due to which the root of tail appears prominent and elevated. The flanks appear hollow with tense and pendulous abdomen. The haunches appear wider.

The gait of cow at full term is sluggish. There is partial anorexia, rumination becomes irregular, pulse and respirations are accelerated but there is a drop in body temperature by  $1^{\circ} - 2^{\circ}\text{F}$ .

The mare has an anxious look with whisking of the tail, sweating in the flank region, exhibiting colicky type of symptoms, frequently sitting down and getting up. Waxing of teats is noticed 24-48 hours before foaling with prominent vulvar oedema and relaxed pelvic

ligaments. The vaginal mucus membrane is of gummy consistency.

In goats the symptoms of approaching parturition are the same as in cow with the exception that udder development is not so marked. During the first stage of parturition symptoms of abdominal pain and discomfort may not be evident especially in pluriparous animals. The doe will show anorexia, stand with an arched back and raised tail, strains occasionally and ruminate irregularly. It has been reported that ewes approaching parturition showed a drop in body temperature  $1^{\circ}$  to  $1.5^{\circ}\text{C}$ . in 24 to 48 hours before parturition. But this fall in body temperature is of little practical value in predicting the onset of parturition. About 69.5 per cent of foetuses are in normal anterior presentation with extremities extended. The second stage of labour in goats is usually completed in about an hour (range 0.5 to 2 hours), a slightly longer if twins or triplets are present. The umbilical cord in the ovine foetus is ruptured as the foetus passes through birth canal. The length of time required for the expulsion of foetal membranes is normally  $\frac{1}{2}$  to 8 hours.

Parturient ewe and goat bleat and the development of mammary glands is not marked. The sow grunts and prepares parturition bed.

The bitch and the cat become restless, nervous and may vomit. They seek for a dark quiet place. The vulval lips become oedematous with mucoid discharge noticed particularly in multi gravida.

All symptoms indicated above are deceptive and vary between individuals and even between the consecutive parturitions. These symptoms do not indicate exact time of parturition. A very guarded opinion has to be given when a Veterinarian is called upon to predict the exact time of parturition.

## STAGES OF PARTURITION

### First stage (Dilatation of cervix)

This stage is characterised by dilatation of cervix and rhythmic contractions of longitudinal and circular muscle fibres of the uterus. Prior to this stage, the uterus is relatively quiescent, but towards the end of gestation period, the contractions increase in quantity and quality due to accumulation of a contractile protein-actomycin. With beginning of these contractions the foetal fluids and membranes are forced against the cervix which causes relaxation. Each contraction or labour pain lasts for about twenty seconds or so recurring at every 15 minutes. As the time passes, they tend to increase in frequency, duration and intensity. Later, contractions may occur every few minutes. It is pertinent to note that any disturbance at this stage is likely to inhibit these contractions, thereby slowing the process. This is due to the fact that in such animals, adrenaline is secreted which blocks the action of oxytocin — a key force for parturition (Cannon, 1953). With increased contractions there is a progressive dilatation of cervix.

false water bag. The chorioallantois ruptures and allantoic fluid flows from the vulva.

After the rupture of chorio-allantois, the amnion is pushed into the cervix and the foetus passes through the cervix and vagina. On entry of foetus in the pelvis, reflex stimuli lead to straining or labour which occurs on account of the contractions of abdominal muscles and diaphragm, with the glottis closed and the second stage of parturition begins.

In cow and ewe this period may last for 1/2 to 24 hours, with an average of 2 to 6 hours (Table 9). Prolongation of this stage over 6 hours indicates that there is some difficulty with regard to presentation. In the mare, this stage lasts for about 1 to 4 hours. In bitch, sow and cat this stage lasts for 2 to 12 hours.

over the metacarpal bones which forms a wedge to dilate the vagina. Once the head has cleared the vulva, the dam takes a little pause to allow the body tissues to get accustomed to this dilatation. By this time the chest has appeared in the pelvic cavity. The hips usually follow through the birth canal fairly rapidly and the further process is facilitated due to the weight from hanging parts of the foetal body. The hind limbs may remain in the passage until the foetus or the dam moves (Fig. 15 A, B, C, D, E, F, G).

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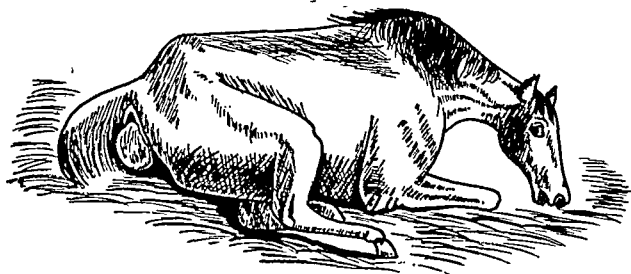


Fig. 16: Parturition in mare. Note the sternum recumbency.



A



B





D



E



F



G

Fig. 15 D E, F, G. Sequential first and second stages of parturition in a Dangl cow.

Table 8  
STAGES OF PARTURITION IN BUFFALOES

Breed	Intensity	Time required in minutes				Reference
		Stage-I	Stage-II	Stage-III	Total	
Murrah	Intense	38.43	16.26	219.20	258.0	Roy & Luktuke (1962)
	Normal	61.05	18.00	240.15	306.7	
	Weak	107.30	—	—	240.15	
Murrah	Intense	49.4 ± 4.3	7.3 ± 1.0	50.0 ± 4.3	270.0 ± 23.2	Singh et al (1966)
	Normal	111.6 ± 6.6	9.1 ± 1.8	282.3 ± 21.9	396.8 ± 23.1	
	Weak	211.9 ± 14.9	11.3 ± 1.9	266.2 ± 33.6	480.9 ± 44.8	
Nagpuri (Berari)		38.57 ± 7.4	185.7 ± 4.7	392.75	461.13	Pargaonkar (1969)

umbilical artery retracts along with the urachus into the abdominal cavity. In the cow, the umbilical cord is short. On expulsion of foetus in reclining position the dam tries to rise up when the cord ruptures, however in some cases due to the strong and elastic structures the rupture does not occur. The dam licks the foetus clean, gnaws at the cord and sets free the young one, preventing bleeding from the naval. The umbilical vein collapses and the blood drains out and so also the fluid in the umbilical cord. The umbilical cord dries up and the umbilicus heals under a scab in 7 to 21 days.

### Third stage (expulsion of placenta — membrane stage)

Expulsion of membranes is rapid in healthy animals. The process involves hormonal and mechanical factors. Early expulsion of foetal membranes is essential since they decompose, become easily infected and may set up metritis.

Expulsion of membranes is associated with uterine contractions. Due to the influence of oestrogen and oxytocin peris-

taltic contractions commence at the cornual apex leading to inversion of chorio-allantois. The capacity of the uterus is reduced. The blood in the endometrium is diminished. The uterine artery is collapsed, becomes thick and fremitus disappears. This leads to dilatation of endometrial crypts, placental villi become small, shrunken and get detached from crypts. Fatty degeneration of the placentome also occurs. The placenta thus gets separated from the uterus.

Uterine contractions aided by those of diaphragm and abdominal muscles force out the inverted chorioallantois through the flaccid os to the vagina. The weight of the membranes hanging from the vagina further assists in the separation. Contractions of vagina are not of much significance in this process. Haemorrhage does not usually occur.

The time required for this stage varies with species and individuals. In cows and buffaloes due to its attachment to over 100 cotyledons, it takes longer time for separation — about 0.5 to 8 hours. Retention of foetal membranes is therefore commonly observed 5-15% (Salis-

bury and VanDemark, 1961), 10% (Mc Donald, 1969) and 12.6% in buffalo (Singh and Rao, 1957). Details are given in Tables 8 & 9.

Expulsion of foetal membranes is extremely rapid in mares requiring about 1/2 to 3 hours. The separation of membranes is rather rapid on account of diffuse placentation and hence retention is uncommon. It is common to observe a foal born with in-tact amnion through which the respirations of the foetus can be noticed. In ewe and goats the expulsion occurs within 2-3 hours. It differs in sows occurring within 10 to 15 minutes. The process is also irregular. The membranes may be expelled individually or in 2 to 3 masses during the labour (Jones, 1966). In the bitch and cat, membranes are expelled after each foetus, the last one may occasionally be retained in the cornua.

Some animals such as bitch, cat, sow, ewe, goat, cow and buffalo may devour the placenta. This act is called as 'placentophagy.' In bitches it may be due to protein hunger. The expelled membranes should be removed because ingestion may cause choke, indigestion, diarrhoea, toxæmia and other digestive disturbances. Mare is an exception since she does not eat placenta. This has been ascribed to the behavioural adaptation by the dam to predation on the newborn (Fraser, 1968).

### Total duration of parturition

The total time required for parturition varies between the species and individuals (Table 9). Cows and buffaloes usually take about 1 to 2 hours since the uterine contractions are not powerful. In mares, the act is fairly rapid requiring about 5 to 15 minutes as the uterine contractions are powerful and continuous. Ewes and goats usually re-

quire 0.5 to 2 hours for the act, whereas in the sow, bitch and cat the interval between the foetuses is about 10 to 30 minutes, the whole process being completed in 1-2 hours. This will however depend upon the number of foetuses.

The three stages of labour in the cat are similar to those in the bitch. At the onset of the first stage the rectal temperature drops very sharply. The cat becomes uneasy, anxious and seeks for a dark quiet place. It will be seen that the cat makes attempts to prepare the place. The first stage lasts for about 12 to 18 hours or even more.

In the second stage there is straining and expulsion efforts are marked. There is protrusion of the amniotic sac and a kitten follows which is expelled immediately. At birth the kitten may or may not be surrounded by foetal membranes. In case the foetus is not accompanied by the foetal membranes, the same is expelled before the expulsion of the next kitten. In cats retained afterbirth is rare. In case there is retention of placenta it is usually expelled in about 8 to 12 hours. If retention is beyond 12 hours administration of oxytocin may be found beneficial.

The third stage is characterised by uterine rest which follows after the expulsion of every kitten. The rest periods vary from 10 to 60 minutes between kittens. However at times it is not unusual for two kittens to be born within interval of few minutes between them. Such paired expulsion of foetuses is usually followed by longer rest periods.

### Occurrence of Parturition

Majority of parturitions occur during day time as 87.6% in Gir (Phatak, 1965), 100% in Rathī (Sonawane, 1969) and 87% in Haryana (Subramanian, 1961) cows. Amritkar *et al*, (1979) reported

Table 9

## STAGES OF PARTURITION IN COWS

No.	Writer	Breed/Species	1st Stage	2nd Stage	3rd Stage	Total Time
1	2	3	4	5	6	7
1.	Williams (1943)	Heifer	1.4 hr.	1.4 hr.	9.4 hr.	—
2.	Williams (1943)	Cow	3.2 hr.	3.2 hr.	14.0 hr.	—
3.	Roberts (1956)	Cow	2.6 hr.	0.5 hr.	0.5-8 hr.	—
4.	Wright (1950)	Cow	—	—	—	4 hrs.
5.	Clegg (1954)	Cow	0.5-2.4 hr.	0.5-4 hr.	0.5-8 hr.	—
6.	Subramanian (1961)	Hariana	95 $\pm$ 75 m	21.23 $\pm$ 1.4 m	278.14 $\pm$ 15.3 m	375.63 $\pm$ 17.5 m
7.	Phatak (1965)	Gir	40 $\pm$ 10 m	39.40 $\pm$ 4.53 m	274.18 $\pm$ 18.49 m	354.20 m
8.	Putbey (1965)	Danpi	113.08 m	20.23 hr.	3.04 hr.	5.27 hr.
9.	Fattle (1967)	Khillar	76.0 m	31.1 m	180.9 m	5.55 hr.
10.	Somawane (1969)	Rathi	60.43 m	8.93 m	284 m	353.36 m
11.	Dange (1969)	Gir	hr. 1.36 32 S.	36m 46-S	4-hr. 58-m 27 S	6-hr. 41 m
12.	Poochhe (1969)	Gir	78.8 m	17.47 m	353.3 m	559 m
13.	Kadu & Kulkarni (1975)	Sahiwal	128.37 $\pm$ 6.07m	17.56 $\pm$ 1.70 m	246.48 $\pm$ 7.60 m	378.16 $\pm$ 9.48 m
14.	Amthar et al (1976)	Gir	63.6 $\pm$ 3.48m	25.20 $\pm$ 1.67m	253.00 $\pm$ 37.08m	342.00 $\pm$ 35.3 m



calving in Gir cows as 41.7% during early morning hours and 50% during rest of the time in which 9.3% occurred during night hours. The factors involved in this circadian rhythm of parturition are not clearly understood.

### Intra-uterine presentation of the foetus

In the cow the foetus lies to a large extent in one uterine horn on its sides. Prior to the beginning of the uterine contractions the young one rotates to the upright position with its nose and forelimbs directed towards the vulva. In the cow, length of foetus after 60 to 90 days of gestation is much more than the gravid horn. The intra-uterine position of the foetus is such that the umbilicus of foetus is in apposition to lesser curvature and dorsum of foetus lies along the greater curvature of uterus. In mares, sow, dog, and cat the foetus rests with its dorsum against the abdomen of its dam in dorso-pubic position and the foetus has to rotate before entering into the pelvis.

In the mare the foal is carried in the body of the uterus and lies on its back with limbs flexed, the neck bent and the mouth touching the breast. Prior to parturition it rotates on its long axis as in the sow and the limbs are extended due to foetal movements, failing which dystokia is likely to occur.

In ewes and goats similar changes occur as in the cow. In poly-tocus species such as sow, bitch and cat, the diameter of the foetus is much smaller than the pelvis and parturition is comparatively easy. The foetuses may be arranged in the uterine cornua with the heads facing either towards the cervical or the ovarian end.

### A. PRESENTATION OF THE FOETUS

Presentation is defined as the relation of the spinal axis of the foetus to that of the mother. The foetus together with its membranes has the form of an oval body which passes to the inlet by two possibilities.

1. When the long axis of the foetus is parallel to the long axis of the dam — longitudinal presentation.
2. When the spinal axis of the foetus is at right angle to that of the dam — Transverse presentation.

In the longitudinal presentation when the anterior part of the foetal body — head, forelimbs or chest is directed towards the pelvic inlet it is termed as anterior presentation (Fig. 17). When the hind limbs and the tail are presented towards the pelvic inlet it is termed as posterior presentation. In the transverse presentation when any portion of the back or loins face the pelvic inlet it is known as dorsal or dorso-lumbar presentation whereas when the sternum or abdomen of the foetus are presented at the pelvic inlet, it is termed as ventral or sterno-abdominal presentation.

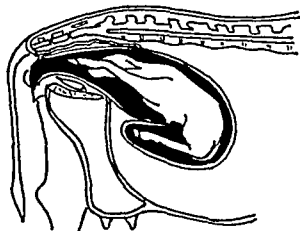
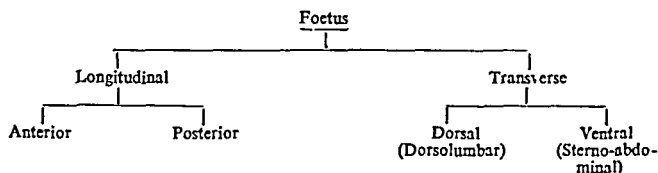


Fig. 17: Anterior longitudinal presentation of foetus.

The presentations can thus be tabulated as under:



## B. POSITION OF THE FOETUS

The position of the foetus includes the relation of the dorsum of the foetus in longitudinal presentation or the head in the transverse presentation to maternal pelvis — sacrum, right left ilium and the pubis.

Presentation and position of the foetus have all possible variations of the manner in which the fetus may enter the pelvic inlet at parturition, the important ones are as under:

<i>Presentation</i>	<i>Positions</i>
Anterior, Longitudinal	Dorso-sacral Right Dorso-ilial
Posterior, Longitudinal	Left Dorso-ilial Dorso-Pubic

is required. In cows 95% of the calves are born in anterior presentation, 99% in mare, 95% in ewe and 70% in bitches (Williams, 1913; Benesch, 1952; Vande plassche, 1957 and Arthur, 1964). In sows, this incidence is 51% (Benesch, 1952 and Arthur, 1964), 61% (D'zuik and Harmon, 1968) and 75.7% (Jones, 1966).

The posterior longitudinal presentation in unipara may lead to dystokia. But in multipara it may be considered normal since the head of the young one is short and seldom deviated. The limbs are also small and flexible and rarely cause any obstacle.

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# Chapter 5

## Postparturient care of the dam

In normal parturitions no care by way of medication is necessary. However, hygienic precautions are most essential. Calving boxes or standings should be clean, dry, and well bedded. The soiled bedding should be changed often and disposed of hygienically. A careful watch on the dam is necessary during the first 24 hours after parturition to note as to when placenta is expelled and if there is profuse straining, hemorrhage and prolapse either of vagina or uterus. In such cases prompt attention is necessary. The expelled afterbirth should be removed and buried or burnt.

The soiled hind quarters and perineum including udder should be cleaned and dried. The young one should be helped to suckle and the colostrum

prompt attention. In heifers failure of milk let down may occur as a result of oedematous tense udder. This may be relieved by injection of oxytocin or pituitrin.

In mare profuse perspiration is an indication of exhaustion and the condition should be diagnosed and treated accordingly. Bran mash gruel and greens may be given in plenty.

In ewes and goats no postparturient care is necessary. However in case twin lambing is suspected the first born should be fed with diluted cows milk until the completion of lambing.

Sows often suffer from exhaustion after farrowing due to large number of piglets born at a time. She therefore requires plenty of nourishing and easily

withdrawal of concentrates, and reducing quantity of water. Injections of 15 to 20 mg. of oestrogens in large animals and 5-10 mgm for bitches are recommended for drying.

### Care of the Newborn

The well being of a new born depends upon how a young one gets an opportunity to develop in a disease-free womb during prenatal life. During intra-uterine life the foetus is nursed entirely by the dam and in the course of progressive development, demands made by the foetus are very heavy. The greatest adjustments a new born has therefore ever to exercise is at birth.

This is the most critical stage in the life of a new born when it is exposed to various external stimuli. The young one has all the potential for independent existence but it has to bring into function all the systems of the body.

At birth the maternal connections are severed due to rupture of umbilical cord resulting in cessation of nutrient and oxygen supply to the foetus. In certain cases, the lungs may not start functioning at birth. This condition is known as syncope or suspended animation and usually follows dystokia. All attempts should be made to induce artificial respiration.

#### 1) Onset of Respiration in the new born

It is very necessary that during parturition an attendant should be close by in order to remove the foetal membranes, if any, and clear up the mucus around the muzzle and nostrils. In bovines the young one is usually born free of foetal membranes but in mares it is not uncommon to find a foal born with intact amnion and even the respirations could be noticed through this membrane. In such a case it is essential that

the foal should be set free by cutting open the membranes.

Various factors are responsible for the initiation of respirations such as increased Carbon-di-oxide-tension in foetal blood, increased hydrostatic pressure in foetal circulation, low level of foetus than that of dam, removal of foetal membranes over the nostrils or combination thereof. In case the respirations are not initiated, following procedures should be adopted to stimulate the respirations: removal of mucus from the nostrils, keeping the new born on hay in an inclined position with head lower than that of hind quarters, a brisk rub all over the body with straw, hay or gunny piece, forceful movements of the head, use of respiratory apparatus and alternate compression of the chest. Respirations can also be stimulated by drawing out the tongue to and fro, blowing air into the nostrils, moving the fore limbs, splashing cold water on the body and by applying aromatic spirits to the nostrils. Intracardiac injections of adrenaline, coramine have been found successful. Alternate dipping in hot and cold water may be of some use in pups and litters.

According to Too *et al*, (1967), the heart beats increase from 54 to 60 to 108 to 216 per minute in new born equine and 70 to 108 to 96 to 120 per minute in bovine foetus. This work was based on electrocardiographic studies.

#### II) Prevention of umbilical infection

In normal course of parturition in any species, the umbilical cord usually ruptures at about 2" from the umbilicus by laceration, linear tension and over stretching. Under natural condition this requires no special attention. But under unhygienic conditions prompt attention

the cord is not ruptured it should be ligated at about 2" from the umbilicus severed with scissors and the stump should be cleaned with cotton swab soaked in dettol or any other antiseptic. To this stump various antiseptics can be applied such as tincture of iodine, 5% tannic acid, 5% salicylic acid in 70% alcohol and 2% copper sulphate. Astringent powders such as alum, tannic acid or turmeric powder can also be dusted on the stump. The treatment may be repeated twice or thrice a day for a couple of days after birth. This treatment should be coupled with parenteral administration of broad spectrum antibiotics in order to prevent navel ill infection. In case of foal, tetanus antitoxoid may be injected simultaneously with an advantage. The importance of clean hygienic, well bedded byres is essential for the new born and parturient dam.

should clean the mucus all over the body with the help of wisp of hay or a piece of gunny cloth and in case, the foal or calf is weak, it should be brought near the teats of mother and induce suckling. Restraint may be necessary to control the dam, particularly when the udder is tender.

The young one should get colostrum within first two hours after birth since it contains antibodies which give passive immunity and is responsible for preventing clinical E. coli enteritis and white scour. It has laxative property and is rich in proteins, fats, minerals and iron than ordinary milk. According to House (1968) cited by Roberts (1971) the amount of Gamma globulin secreted through colostrum is directly proportional to the susceptibility of disease and death. A field test devised by him consists of adding 0.5 ml. calf serum to 10 ml. of 22.5% ammonium sulphate solution, the degree of turbidity indicating the level of Gamma globuline

born with foster mother's milk or synthetic preparations such as calf starters. The foster mothers usually adopt the new born orphan with ease. This is particularly so in cows, dogs, sheep and sows. However it may be necessary in some cases to smear the new born with foetal membranes or genital discharges or putting the hide of the dead offspring or wool of the ewe from the would be foster mother. Powdered milk can be used for raising orphan foals. The young foal may not relish cow's milk and chronic diarrhoea usually sets in. Pigs, pups and kittens tolerate cow's milk very well. The new born should be fed every 3 to 4 times a day during the first week. Feeding may be done by nipple bottles, bowls or buckets. There is a good axiom that "it is difficult to starve a young one but very easy to overfeed it." The amount of milk to be fed should be determined on the weight of the calf. Commercial calf starters and pig starters are nowadays available, which contain antibiotics and vitamins. The young one should be induced to take greens and solid food as early as possible. Simultaneously, the amount of milk should be reduced proportionately. The new born should be examined carefully to ascertain that all the natural orifices are patent and if not, a timely surgical intervention is necessary.

Gentle exercise is necessary for the foal and calf a few days after birth. A calf box is usually sufficient.

Calves which are raised artificially on milk fed through buckets lick each others body coat after finishing the quantum of milk, thus leading to formation of hair balls in their stomach. To prevent this, their mouth should be washed with warm water and tender hay mixed with mineral mixture or salt be kept in their mouth to satisfy their suckling in-

stinct. It is also necessary that calves should be housed according to their age groups. Slippery floors are dangerous and therefore saw dust, sand or hay may be used to prevent slipping. A vaccination programme should be instituted according to age group.

#### iv) Anomalies at birth

A new born is occasionally seen with certain conditions which may have a congenital or hereditary origin. These include atresia ani, cleft palate, undershot jaw, contracted tendons, blindness, cerebral hypoplasia, umbilical and scrotal hernia, cryptorchidism and others. These have been described in chapter on lethal factors and malformations. An isolated incidence may not be of a great concern in a herd but their frequent occurrence is suggestive of inbreeding. Certain cases may recover but others require surgical or medicinal treatment.

A calf may be born with retained meconium, persistent urachus and rupture of bladder. Occasionally meconium may be retained in the form of hard pellets especially in foals. In such cases the new born shows colicky symptoms and lack of appetite. The logical treatment would be to give enema of saline, soap and water or glycerine, or castor oil to be repeated if necessary. Retained pellets should not be withdrawn manually with force.

Persistent urachus is usually encountered in a new born foal but rare in calf and is characterised by continuous dribbling of urine through urachus which keeps the surrounding skin moist. Cauterizing the affected part with tincture of iodine, 10% formalin, copper sulphate and parenteral administration of antibiotics in combination for a period of 8 to 10 days is found beneficial.

Rupture of the bladder is of rare occurrence in new born foals. This is indicated by the enlarged size of the abdomen. The pulse and respirations are accelerated and the foal appears distressed. Diagnosis can be confirmed by exploratory puncture of the abdomen. Laparotomy and locating the seat of rupture and suturing it coupled with antibiotics and supportive treatment is useful.

#### v) Transuterine infection

It is likely that foetus may contract infection from the uterus at parturition

or even before birth. Transuterine infections of the foetus are rare compared to the infections contracted by the new born. The type of parasites transmitted in utero from the dam to the foetus are mentioned in table 10.

In addition to this, the foetus may contract various bacterial and viral infections through the dam, which are also shown in the same table.

#### vi) Neoplasms of new born

Various types of tumours including neoplasms of the gonads have been recorded in the new born. These are rare

Table 10

Sr. No	Species	Organism	Parasitic
		Bacterial and viral	
1.	Cattle	<i>Brucella abortus</i> , <i>Leptospira</i> sp., <i>Listeria monocytogenes</i> , <i>E. coli</i> , <i>Streptococci</i> , <i>Toxoplasma gondi</i> , <i>Aspergillus</i> sp., <i>Salmonella</i> sp., BVD MD virus, <i>Micobacterium para tuberculosis</i> .	<i>Neoascaris vitulorum</i> , <i>Theileria annulata</i> , <i>Anaplasma marginale</i> , <i>Cysticercus bovis</i> .
2.	Horse	<i>Streptococcus zooepidemicus</i> , <i>E. coli</i> , <i>Actinobacillus equi</i> , <i>Leptospira</i> , <i>Salmonella abortus</i> <i>equi</i> , Herpes I virus	<i>Babesia caballi</i> , <i>Strongyloides westerni</i> .
3.	Sheep and Goats	<i>V. foetus intestinalis</i> , <i>Brucella mellitensis</i> , <i>Salmonella</i> sp., <i>E. coli</i> , Enzootic abortion virus, <i>Toxoplasma gondi</i> , <i>Micobacterium johnes</i> .	
4.	Swine	<i>Leptospira</i> sp., <i>E. coli</i> , <i>Toxoplasma gondi</i> , <i>Epicephalothrix suis</i>	<i>Strongyloides ransomi</i> , <i>Stephanurus dentatus</i> , <i>Acaris suis</i> .
5.	Dogs and Cats	<i>Streptococci</i> , <i>Staphylococci</i> , <i>E. coli</i> , <i>Pseudomonas aeruginosa</i> , Feline panleucopenia virus, Distemper virus, <i>Toxoplasma</i> <i>gondi</i>	<i>Toxocara canis</i> , <i>Ancylostoma caninum</i> , <i>Dirofilaria imens</i> , <i>Demodex canis</i>



but papillomas of the skin are common in Gir cattle and buffalo calves. Testicular adenoma and ovarian granuloma in ovine foetuses have been reported by Kanagawa *et al.*, (1964). Occasionally, sublumbar lipoma may be observed.

#### vii) Injuries to the new born

During normal parturition the foetus is born without apparent injury, however, in difficult births it becomes necessary to give forced traction on foetus either with hands or instruments. This may lead to fractures of the bones at the site of application of force, for example, the jaw bones, the metacarpus, the metatarsus, digits and ribs. Dislocation of hip may also occur due to forced traction. If the young one remains in the passage for a long time in anterior presentation with head protruding out of vulva for a long time, then oedema of tongue and forelimbs may develop. Appropriate palliative treatment is necessary in such cases.

The other rare conditions observed in foals are neonatal isoerythrolysis, respiratory distress syndrome and convulsions in thorough bred new born foals. These have not been reported under Indian conditions. Persistent bleeding from navel stump in calves and fibroelastosis of heart valves in pups and kittens may also be observed.

#### viii) The diseases of the new born

Certain affections commonly occurring in the early postnatal life of a new born are described below:

##### 1. WHITE SCOUR

This is the most common disease of the young calves and occurs within the first three to four weeks of life. The mortality is higher in the first fortnight. There are two types of infection:

##### i) *E. coli Septicaemia*

This occurs very early in life or even in a day old calf. In acute cases, there is septicaemia and the calf is found dead. The organisms gain entry into the blood stream from the intestine during the first 24 hours of life when the intestinal wall is permeable to the passage of proteins. There are certain virulent strains in coli against which no antibodies can be demonstrated in the colostrum.

##### ii) *E. coli localised intestinal infection*

This condition is usually seen in the first week of life. Foetid diarrhoea is most predominant symptom and the colour of the faeces, varies from yellowish brown to white. There is progressive loss of appetite, dehydration and sinking of eyes.

Treatment includes withholding of milk and parenteral administration of glucose-saline together with oral administration of antibiotics such as streptomycin and sulpha drugs.

#### 2. NAVEL ILL OR JOINT ILL

The infection gains entry into the body through the navel. The mortality is high. In acute cases death is sudden without any specific symptoms. In less acute cases, there is swelling of the navel together with abscess formation. Joints, especially hock or knees are usually hot, swollen and painful. Such acute cases should be treated with parenteral administration of antibiotics. The navel abscess should be treated in the usual manner.

#### 3. CALF DIPHTHERIA

Causal organism of the disease is *Fusiformis necrophorus*. The disease is usually observed in calves 4 to 42 days old.

The clinical symptoms manifested in the affected calves are slobbering and swelling at the side of cheek or in the throat region laboured breathing coughing along with sticky greenish discharge from the nostrils. As the disease progresses the swollen tongue protrudes and there is offensive odour. In later stages of the disease diarrhoea sets in. Majority of cases succumb within a week due to severity. Antibiotic therapy is indicated.

#### 1 CALF PNEUMONIA

Exposure of the calves to inclement weather and damp and cold flooring are predisposing factors. There is respiratory distress nasal discharge coughing and high temperature. Antibiotic therapy is recommended. Buffalo calves are more susceptible to cold as compared

to cow calves. Pneumonia and lung abscesses are of common occurrence.

#### 5 BLOAT

Bloat is usually observed in calves fed on milk or milk starters low in fat. The treatment consists in reducing the intake. Weaning the calf and placing the calf on dry food is advisable.

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# Chapter 6

## Involution of Uterus

After the normal parturition, the expanded uterine bag is expected to attain its original position and size within specified time. This reposition is physiological and the process is termed as involution of uterus. Uterine involution has a great bearing on subsequent fertility.

### Involution of uterus in the Cow

#### NORMAL SIZE AND LOCATION OF UTERUS

In the non-gravid condition the uterus in the cow can almost be located within the pelvic cavity. The body of the uterus has an average length of 2.5 to 5 cm. and the average length of each horn is about 20 cm. to 37 cm. depending on the breed and size of the animal.

#### Activity of the uterine muscle

The musculature of the uterus shows spontaneous contraction waves, intensity of which varies in different stages of oestrous cycle or during and after parturition. The contractions are predominantly peristaltic in nature but may at times be antiperistaltic. The contractions originate from the apex of the uterine horn and pass towards cervix. Uterine contractions may occur in pregnant and non pregnant horns upto about two to three months of gestation. From the mid-term however, there is complete

quiescence of the muscular activity of the uterus.

#### Changes in the size of the uterus during pregnancy

The uterus undergoes marked enlargement during pregnancy. The smooth muscle cells show a marked increase in size and possibly also in number as a result of connective tissue differentiation. Considering the size, length and weight of the fullgrown foetus and the weight of the amniotic and allantoic fluids the uterine musculature particularly of the gravid horn has to stretch to a considerable extent. It is interesting to note that the development of uterus during pregnancy is gradual and spread throughout the period. Whereas, in the case of involution, the onset of regression is sudden and rapid. In certain species it is just a matter of few days.

#### Process of involution

The normal uterine involution is an aseptic process in which various cells originating from maternal placenta, inter-caruncular mucosa and blood are dissolved and degenerated. The process is exceedingly rapid in the initial stages. There is rapid and progressive loss of Collagen in the muscle cell cytoplasm and ground substances produced

during the growth of uterus at the time of development of foetus.

Complete involution is judged on rectal palpation by: i) return of the uterus to its normal location in the intra-pelvic cavity or near pelvic brim. ii) Normal and approximately equal size of uterine horns. iii) and attainment of normal uterine tone and consistency. The horn which was gravid will assume comparatively bigger size.

In the cow and buffalo, majority of the pregnancies occur in the right cornua. As such, the cornua which is subjected to large number of pregnancies will assume bigger size after every calving, compared to the non-gravid one. Therefore, disparity in size of the horns even after complete involution is usually felt on palpation. Even after complete involution, the walls of the horn that was gravid will still remain slightly thickened and the horn never undergoes complete involution to its pre-gravid size. Involution of the uterus following each succeeding pregnancy is never complete i.e. the organ does not return to its normal pre-gravid location and size. Thus the uteri of animals, which have passed through several pregnancies assume much larger size than

parturition, the waves occur only every 8 to 12 minutes. By about 72 hours, the rhythm is very irregular and weak. From 4th to 8th day, there are only irregular undulations of the horns (Benesch, 1952). The continued peristaltic contractions of the uterus from apex of the horn towards cervix help in the expulsion of the placenta. It is only after expulsion of afterbirth that the uterus starts contracting very rapidly and the normal process of involution begins. At this stage rectal palpation will give a feel of a very large loose bag with elevations due to hypertrophied caruncles. The muscular contractions constrict the uterus and produce a thickening of the walls and in this way facilitate involution by expelling material that may be left in the lumen of the organ. The contractions of uterus also produce resistance against the large in-flow of blood which is now no longer required.

Rasbech (1950) observed that the maternal placenta involuted by the disappearance of the caruncular stalk through vaso-constriction, by the dissolution of the uterine caruncles by fatty infiltration and dissolution and detachment of the entire superficial layer of the caruncles with the formation of uterine

post partum and by the 20th day a small elevation of 0.5 cm. remains on the surface of the endometrium.

### LOCHIA

The uterine lochia consists of mucus, detritus and blood. Within first 48 hours after parturition, the amount of lochial discharge voided is the greatest (1400 to 1600 ml.). By about 8th day after parturition, it is decreased to 500 ml. and by 14 to 18 days it is only a few ml. In the primipara, the quantity is about 500 ml. In pluripara quantity may extend from 800 to 2000 ml. The discharge is of amber to red in colour and odourless. It may also be of light yellow brown colour. Within about 24 hours after parturition there is hyperleucocytosis which lasts for 2 to 3 days. The emigration of leucocytes takes place from the cervical mucosa and the leucocytes are mixed with the mucus giving turbid appearance to the discharge. The aseptic process of involution is further continued without admixture of any larger amounts of leucocytes than those corresponding with the blood stream. In about 9 to 10 days postpartum, increased amount of blood is found mixed with lochia. The detritus is light chocolate brown in colour and is often yellowish. The decidual detritus disappears in 9 to 10 days post partum, after which an increased admixture of blood generally appears, culminating on the 12th day and decreasing thereafter. This blood originates from the haemorrhage on the surface of caruncles.

### Time required for involution

If everything proceeds normally, the uterus becomes definable, on palpation per rectum, in about 8 to 10 days after parturition both in primiparous and pluriparous cows. In primiparous

cows, the involution of uterus is usually completed in 18 to 20 days and in pluripara, it is usually completed in three to four weeks. This is however, based on return of the uterus to its intrapelvic location and reduction of size more or less close to its pre-gravid status. It is not possible to ascertain the state of the endometrium by rectal palpation alone.

Casida and Venzke (1936) reported that involution of uterus in Holstein cows was completed on an average of  $26.2 \pm 1.0$  days. Rasbech (1950) reported that in primipara and secundipara involution was complete within 18 to 20 days and 20 to 25 days in pluripara. Buch *et al.* (1955) considered that a relatively low conception results obtained at the first post partum oestrus which falls within 60 days are presumably due to incomplete involution of the uterus. He reported a period of 42 days in primipara, 50 days in pluripara and an average of 47 days for complete involution of uterus in cows. The difference in the involution interval between primiparous and pluriparous cows was highly significant. Significant differences were also observed amongst seasons being shortest in summer and autumn. Benesch (1952) mentioned that the cervix in the cow contracts more rapidly than the uterus. After 24 to 30 hours following normal parturition, it is not possible to pass the hand through cervix and by about the 4th day it is difficult to insert even two fingers. Subramanian (1961) recorded in Haryana cattle an average period of involution as  $33.5 \pm 1.0$  days. He further observed that the non-gravid horn involuted earlier than the gravid horn. Purbey (1965) reported in Dangi cows an average of 22.14 days for completion of uterine involution. He also observed

that the non-gravid horn involuted earlier by 4.2 days. Mateev (1966) reported an average of 45 days for completion of uterine involution. Morrow *et al*, (1966) reported an average period of 25 days. Marrion *et al*, (1968) reported an average period of uterine involution as 34 days in primiparous cows and 41 days in pluriparous cows. Francis (1969) reported an average period of uterine involution in Sindhi cows as 36.27 days. Sonawane (1969) reported an average period of uterine involution as 36.72 and 42.70 days in Rath and Gir cows respectively. Wagner and Hansel (1969) observed that the uterine mucosal epithelium is re-established in most of the normal cows within 30 days post partum. Morrow *et al*, (1969) studied 204 post partum cows and reported an average period of 25 to 30 days for uterine involution to occur. They further reported the conception rate which varied from 47.6 to 88.9% which was highest between 90 to 120 days post partum.

The average interval between parturition to complete involution of the uterus was  $35.1 \pm 1.2$  days in 20 Gir cows following normal calving. Non-gravid horn involuted earlier (27.2 days) than the gravid horn (32.3 days). The uterus was observed completely intrapelvic about 24 days after calving. The estimated diameter of cervix, gravid horn and non-gravid horn of the uterus at 6 weeks after calvings was 2.9, 2.4 and 2.3 cm. respectively. Uterine involution in cows with 1-4 calvings and 5 or more calvings had taken at 32.3, 35.3 and 36.0 days respectively. The discharge of uterine lochia was observed on an average for about 12 days (Rangiah, 1971).

## Factors influencing involution of Uterus

### 1) SPECIES

The period required for complete involution of uterus varies from species to species. It is interesting to note that swamp Beaver can be served on the same day of parturition with good conception results indicating that the time required for involution of uterus is just a few hours (Lagerlof, 1969).

### 2) CALVINGS

Involution of uterus in primipara is rapid and earlier than in pluripara (Morrow *et al*, 1969).

### 3) SEASON

Season has also influence on the process of involution. Buch *et al*, (1955) found that cows calving during winter, spring, summer and autumn required 51, 47, 42 and 44 days respectively for complete involution to occur. Marrion *et al*, (1968) found that the average interval to uterine involution for all seasons of the year was  $34.01 \pm 5.8$  days for primipara and  $40.59 \pm 6.4$  days for pluripara. The interval varies from 30.4 days in spring to 36.5 days in autumn for primipara and from 37.8 days in summer to 41.8 days in winter for pluripara.

### 4) TYPES OF PLACENTA

The rapid involution of uterus in the mare is considered to be due to diffuse type of placenta. The zonary type of placenta in dog and cat does not interfere with the process of involution.

### 5) BREED

The period of involution is more in the milch breed as compared to beef or draught.

### 6) ABNORMAL CALVING

Buch *et al*, (1955) observed that abnormal calvings increased the period of involution.



## 7) RETAINED PLACENTA

Retained placenta acts as an impediment and is usually followed by metritis and endometritis which delay the involution.

## 8) METABOLIC DISEASES

Milk fever, Ketosis and other deficiency diseases result in delayed involution.

## 9) CYSTIC OVARIES

Cystic ovaries following parturition delay the involution.

## 10) RETAINED LOCHIA

Involution is delayed in case the lochia gets locked up in the lumen of uterus.

## 11) TWINS AND MULTIPLE BIRTHS

Roberts (1956) reported that retention of placenta occurred in 70 to 80% twin calves which ultimately resulted in delayed involution.

The incidence of dystokia at the termination of twin pregnancy is much higher than single births. In twin pregnancy the retention of afterbirth is chiefly due to the atonicity of distended uterus.

## 12) SUCKLING

The rate of involution of uterus is faster in cows in which there are suckling calves than the ones in which weaning at birth is practised.

Normal involution usually takes the course of an aseptic process. This is very critical time and if hygienic conditions in the stables are not upto the mark, puerperal infection is likely to occur through various sources which may result in massive bacterial growth in the uterine lochia. Infection with *C. pyogenes* is the most frequent. In such cases the lochial discharge may become purulent, turbid and offensive.

At later stages the discharge may become whitish or yellowish white. Infection with *E. coli* may give the discharge a greyish white appearance with white streaks. The Staphylococcal infection causes considerable changes in the lochial discharge giving greyish white purulent appearance. Mixed infections may cause considerable changes and the lochial discharge may appear normal to purulent.

## 2. Involution of uterus in buffalo

Sane and Desai (1960) reported their observations on involution of uterus on 108 calvings in Murraha buffaloes as 20.05 and 21.10 days in primipara and pluripara respectively. Seasonal variations were markedly noticed viz. in summer 17.8, rainy season 23.7 and winter 20.10 days. It was found that in buffaloes in which involution was completed within 26 days after calving reached their peak milk yield in good time (2 to 3 months) whereas cases in which there were complications due to puerperal infections, involution was invariably delayed with marked drop in the milk yield. Luktuke and Roy (1962) studied involution of uterus in 76 Murraha buffaloes and reported the average interval as  $39.3 \pm 1.2$  days. The non-gravid horn involuted 11.4 days earlier than the gravid horn in 88.5% cases, whereas gravid horn required 31 to 45 days to involute in 58.6% cases. The rate of involution was rapid upto 14 days post partum and then decreased gradually being considerably slow after 21 days. The number of calvings had the significant effect and required 42.4, 35 and 40.3 days for those animals in groups of 2 to 4, 5 to 7 and 8 and more lactations respectively. From their studies it appeared that the uterine in-

volution has a significant influence on post partum conception rate but it was not affected by the seasons.

Sane and Desai (1960) observed in their study of buffaloes that the colour and consistency of the lochial discharge changes from day to day. Within first 24 hours there is admixture of pure blood. On the 2nd and 3rd day the discharge is more of chocolate colour looking turbid due to admixture of leucocytes. On the 4th and 5th day, it changes to chocolate brown to dirty amber colour and later it becomes light grey tinged with occasional streaks of blood changing to whitish yellow and of albuminous consistency. In the normal course the discharge is usually found to have ceased within 2 to 3 weeks and very negligible thick brownish mucus is passed at the end of third week. After two weeks the discharge becomes very scanty. Luktuke and Roy (1962) further observed that the colour of lochial discharge in buffaloes was brown to yellow.

#### **Involution of uterus in Ewe**

Uren (1935) reported that in the ewe, the uterine mucosa was found to have completely involuted by 30 days after parturition.

#### **Involution of uterus in mares**

Andrews and McKenzie (1911) reported that the process of involution of uterus in the mare is fairly rapid with an average range from 13 to 25 days post partum. In the normal course, complete involution of uterus in the mare should occur within 8 to 10 days after foaling at the end of which the mare comes on heat (foal heat) and conceives if served. The rapid involution in the mare is believed to be due to the simple diffuse type of placentation. Lochial discharge may be observed for a week or two.

Arora (1970) reported his findings on the involution of uterus in the mare, based on the interval between foaling and foal heat, to vary from 1 to 18 days in three groups of mares. Of these 83.56% of the horse producing mares, 79.85% of the mule producing mares and 47.38% of the donkey producing mares came in oestrus from 5 to 8 days after foaling. 4.11% horse producing mares, 13.81% mule producing mares and 5.26% donkey producing mares came into heat within 4 days of foaling. This evidently shows that involution is complete within about a week or so.

#### **Involution of uterus in sow**

In the sow, involution of uterus usually occurs within a week after farrowing but at the first symptoms of heat they will not be mated. They will not show the next heat until the litters are weaned off. Lochial discharge is observed for about a week.

#### **Involution of uterus in the bitch**

In the bitch, the uterus assumes its normal position and pre-gravid size in 4 to 5 weeks. Pigmented ring shaped stripes may be observed on the endometrium indicating the sites of placentation, even as late as 3 months post partum. Copious quantities of lochial discharge of dark chocolate green colour and mucoid consistency are voided after parturition. According to Flexner (1934) this green pigment is utero-verdin which comes from the green border of the placenta. By the first week after whelping, the lochial discharge is amber to red in colour and by the second week it should appear normal.

#### **Involution of uterus in the Camel**

In the camel, complete involution of uterus usually occurs in about 10 days



at the end of which calf heat is expressed. Chances of conception are fairly high if served at the first heat. In camel the first post partum heat is termed as 'calf heat'.

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# Chapter 7

## Accidents and diseases incidental to parturition

Post parturient period is one of the most critical periods in the life of a female. The dam has to overcome the stress of parturition, lactation and uterine involution. It is during this period that the animal is exposed to known and unknown types of infections which lead to severe reproductive disturbances resulting in lowered fertility. Some of the most common ailments affecting the parturient dam are described below.

### I. RETENTION OF PLACENTA RETENTION SECUNDINARIUM. RETENTION OF FOETAL MEMBRANES

Retention of placenta is the most common malady during the puerperal period subsequent to parturition and is observed mostly in buffaloes and cows, rarely in other species. The incidence is high in areas with prevalence of *Brucella* infection. Normally the foetal membranes are expelled within 3 to 8 hours after parturition due to hormonal and mechanical factors during the 3rd stage of labour. However, if they are not expelled within 8 to 12 hours they are considered as retained (Roberts, 1971).

#### Incidence

The incidence of retention of placenta varies with the health and hygienic

standards. The Table 11, summarises the incidence of retention of placenta reported in literature.

Table 11  
TABLE SHOWING THE INCIDENCE OF  
RETAINED PLACENTA IN COWS AND  
BUFFALOES

Author	Incidence (%)	Number of observations
Palmer (1932), U.S.A.	11.7	375
Henderson (1938)	12.8	928
Kennedy (1947), Scotland	8.3	431
Boyd and Sellers (1918)	6.4	450
Reimann (1954)	5.0	232
Cohen (1956), Israel	8.9	145,000
Skjerven (1956)	12.0	100
Erb <i>et al.</i> (1958), U.S.A., (Holstein)	10.5	7397
Leech <i>et al.</i> (1960), Britain	3.8	2,700,000
Flegmar <i>et al.</i> Shiplov (1961)	22.0	1254
Ben David (1962), Israel	8.4	21,000
Felix (1962)	10.2	1711
Harvie (1964), Dutch Friesian	19.9	600

at the end of which calf heat is expressed. Chances of conception are fairly high if served at the first heat. In camel the first post partum heat is termed as 'calf heat'.

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## 2 PROGESTERONE

Low level of progesterone favours retention (McDonald *et al*, 1954). The sub optimum balance between oestrogen progesterone level leads to weak contractions of uterus resulting in retention of placenta.

## 13 VITAMINS

(i) Low vitamin A or carotene intake or lack of greens may lead to retention (Ronning *et al*, 1953; Milinac *et al*, 1961; Martinov, 1964; Nicolson, 1965)

(ii) Vitamin B: Low levels of Vitamin B in cotyledons favours retention (Tsolov, 1962)

## 14 OXYTOCIN

Low level of oxytocin favours retention (Miyakawa, 1966)

## 15 MINERAL METABOLISM

Disturbances in mineral metabolism calcium phosphorus ratio and acid alkali balance are responsible for retention (Martinov, 1964). Retention may also be observed in milk fever condition

## 16 NEUTROPENIA

According to Moberg (1956) Winquist (1959) and Moore (1966), the leucocytes reveal maturation inhibition within the blood cells in retention cases

## 17. IODINE

Deficiency of iodine favours retention (Moberg, 1956)

## 18 MILK YIELD

Retention is common in those animals which are high yielders (Flegmatov and Shipilov, 1961)

## 19 UTERINE CONTRACTION

Uterine inertia after parturition and poor or weak uterine contractions due to dropsy of foetal membranes, uterine torsion, twinning foetal gaminum, dys-

tokia, etc favours retention (DeSutter, 1954, Shaw, 1938, Fincher, 1941)

## 20 POISONING

Retention is observed in allergic cases or those with poisoning due to toxic drugs such as extractum felicis (Richter and Gotze, 1960)

## 21 DISTENSION

Excessive distension of uterus may favour retention (Richter and Gotze, 1960).

## 22 INFECTION

Retention of placenta is common in those herds infected with Brucellosis (Fincher, 1941; Boyd and Sellers, 1918; Moller *et al*, 1967). However, Erb *et al*, (1958) and Brands (1966) reported no difference in the incidence of retained placenta amongst Brucella free and Brucella infected herds

The retention is also commonly observed in mould infection (Benedixen and Plum, 1926; Roberts, 1971) and with *Vibrio foetus* (Roberts, 1971) and Tuberculosis (Roberts, 1971)

## 23 UTRINE INVOLUTION

Failure of normal, uterine involution is frequently associated with retention (Roberts, 1971)

## 24 OTHER ORGANISMS

Roberts (1971) reported from his personal observations regarding the enzootic outbreaks of retained placenta due to *Streptococcus dysgalactia*, *E. coli*, *Staphylococcus*, *Pseudomonas aeruginosa*, *Corynebacterium progenes* which may infect the parturient cow at calving and set up metritis and retention. He strongly recommended the regime of retaining parturient cow for 5 to 10 days before and after calving in clean hygienic calving boxes. The incidence in cows has also been markedly reduced at

Table 11 (Contd.)

Author	Incidence (%)	Number of observations
Maijala (1964)	1.9	11,656
Wetherill (1965)	5.9	34,950
Bannerjee (1963), Holland	11.2	2,276
Brands (1966)	5.1	142,076
Ekes Bo (1966), Sweden	8.4	19,264
Moller <i>et al.</i> (1967), New Zealand	1.96	36,000
Vandeplasse & Martins (1961), Belgium	8.0	738
Geyer (1964), Germany	25.00	1,200
Vinattieri <i>et al.</i> , (1947) (Buffaloes)	17.1	512
Chothani (1972)	40.68	28,587
		(of reproductive disorders in buffaloes)

### Aetiology

Retention of placenta denotes the failure of foetal villi to separate from maternal crypts i.e. the lack of placental dehiscence. The causative factors are as under.

#### 1. DURATION OF GESTATION PERIOD

Retention is common in those parturitions occurring much before the expected date (Benesch and Wright, 1951; Cohen, 1956; Moller *et al.*, 1967).

#### 2. SEASON

Incidence was highest in spring compared to other seasons. (Reimann, 1954; Cohen, 1956; Ilancic *et al.*, 1964; Wetherill, 1965). It was highest in buffaloes during August and September (Vinattieri *et al.*, 1945), being the calving season.

#### 3. BREED

The incidence varied from breed to breed. It was more in Holstein and Swedish lowland breeds. (Henderson, 1938; Leech *et al.*, 1960; Ilancic *et al.*, 1964; EkesBo, 1966). It is more in dairy type than in draught or beef type.

#### 4. SEX

Retention is more common with the male calf rather than with the female calf (Rasbech, 1950; Lesbrini, 1964).

#### 5. TWINS

The incidence is more with twin pregnancies (Erb *et al.*, 1958).

#### 6. ABORTIONS, DYSTOKIA, STILL BIRTHS

The occurrence of retention is much more common in abortions, dystokia, still births and other abnormal conditions. (Erb *et al.*, 1958; Leech *et al.*, 1960; Vanderplasse and Martins, 1962; Ben David, 1962; Brands, 1966).

#### 7. RECURRENCE

The retention of placenta recurs to the tune of 20% in the succeeding parturitions (Erb *et al.*, 1958; Brands, 1960; Roberts, 1971).

#### 8. SUCKLING

Retention of placenta is less common in buffaloes that are suckled by calves. (Vinattieri *et al.*, 1945).

#### 9. AGE

Retention of placenta is much more common in primipara and in older cows. (Leech *et al.*, 1960).

#### 10. EARLY POST-PARTUM BREEDING

Retention is observed more commonly in cows bred soon after parturition (Hofstad, 1911; Moore, 1949; Moller *et al.*, 1967).

#### 11. EXERCISE

Confinement to standings or stables and lack of exercise may lead to retention (Martinov, 1964).

In severe cases of longer duration, there is forceful straining, high fever, accelerated pulse, reduced appetite and milk yield. The placenta is discoloured, dry and the animal voids foul smelling discharge which may be blood stained or containing gas bubbles suggestive of clostridial infection. In some cases of retained afterbirth, a portion of placenta hangs outside the vulval lips during sitting posture. This gets soiled due to dung and filth. When the animal gets up, this hanging portion is drawn in the vaginal passage resulting in ascending infection. The placenta is inflamed, oedematous and may even be emphysematous. The portion outside the vagina is discoloured and is soiled with filth, dung and hay. The white blood cell picture shows a marked shift to the left, lymphocytosis and leucopenia are observed (Moberg, 1956). After 24 hours or so the placental maceration starts, the cervix contracts 48-72 hours after parturition and it may be difficult to insert the hand in the cervix.

### Prognosis

Mild cases recover with prompt treatment. However, prognosis is guarded in severe cases because of effects of retention on milk yield and subsequent fertility, delayed involution, chronic endometritis, permanent sterility due to pyometra, perimetritis, salpingitis, ovariitis, severe damage to the endometrium, etc. It is always advisable to take pulse and temperature since increase in pulse rate is the first indication of spreading infection. According to Arthur (1975), the mortality rate should not exceed 12%.

### Treatment

A variety of techniques, drugs and hormones have been advocated in the

literature. The views extend from a thorough treatment to no treatment. In any case, the ultimate aim is that the placenta be removed either manually or by the use of drugs. Thus treatments are grouped into the following categories:

1. Manual removal with or without antibiotics.
2. Only antibiotics local/parenteral.
3. Only hormones parenterally with or without antibiotics.
4. No treatment.

The efficacy of the above four forms of therapy have been compared by several investigators (Ben-David, 1962; Hammerman, 1963; Trainen, 1965; Hatch *et al*, 1968). Manual removal with local administration of antibiotics has been most effective and with a high recovery rate. This has been described in details.

### 1. MANUAL REMOVAL

Before undertaking manual removal, vagina and uterus should be carefully examined, particularly for any rupture. The operator should wear protective clothing to avoid infection. The perineal region of the cow or buffalo should be scrubbed and cleaned with soap, warm water and saxon or dettol. Epidural anaesthesia may be used to avoid defaecation during removal. Tail should be held to one side by the assistant or in absence of him the tail may be tied by rope to the forelimb or horn. The fluid in the uterus, if present, should be siphoned off by a rubber tubing or large cotton swabs. The best time for removal of placenta is 24-48 hours after parturition.

The operation should be done very gently. Each placentome is grasped between thumb and fingers and the two structures be gently separated by pulling

C.B.F. Kandivali (India), due to introduction of clean, hygienic calving boxes (Sane *et al*, 1977).

## 25. TRANSPORT

Transport of high pregnant animals particularly those due for calving leads to retention.

## 26. MALFORMATION

Malformations such as duplex cervix and persistent hymen may lead to retention.

## 27. FAMINE

Retention is more common in areas afflicted with famine or drought.

From a clinical point of view, the aetiological factors can be grouped as under (Lagelof 1969).

- A. (i) Too weak contractions of uterine walls due to hormonal imbalance between oestrogen, progesterone and oxytocin.
- (ii) Clinical or subclinical cases of milk fever in high yielders due to hypocalcaemia.
- (iii) Weakness of uterine wall due to over distention as in twins or foetal giantism.
- (iv) Fatigue or weakness of uterine muscles due to prolonged labour as in dystokia.
- (v) Transportation over long distance, prolonged gestation, old age, etc.
- B. *Too hard fixation between maternal and foetal placentae:*—If the fixation between the foetal and maternal placentae is too strong, the placental dehiscence does not occur. This hard fixation is due to placentitis caused by bacterial, viral, mycotic or protozoan affections. However, retention of placenta

is seldom observed following trichomonad abortion (Roberts, 1971).

- C. *Disturbances in normal separation mechanism:*—In certain cases, separation of placentae does not occur even though there is no evidence of placentitis. Deficiency of Vitamin 'A' and lack of minerals like calcium, inorganic phosphorus, iodine, etc. have been incriminated as causative factors.

- D. *Unknown factors:* In some herds, in spite of the fact that they are free from Brucellosis and Tuberculosis, the incidence of retention will be on the increase but decreases in the succeeding years. Such a phenomenon is difficult to explain.

## Symptoms

The symptoms of retention of placenta are obvious, the conspicuous symptom being the hanging of placenta itself. In some cases however, the placenta may not hang outside. In such cases it must have been dropped and eaten by the cow herself (placentophagy) or removed by canines and rodents. A careful manual search in the vaginal passage is therefore necessary to locate the placenta. In some cases, the entire placenta may be retained inside the genital tract.

In low grade infection, the pulse, temperature, appetite and milk yield may be normal. No foetid smell is detectable around the cow and the placenta may be of normal colour, moist and glistening. A clear mucus string without foetid odour may be seen hanging from the cervix.

### Retention of placenta in the mare

Retention of placenta is rare in mare than in cattle, but it is a serious condition. It is more common in draught mares as compared to pure bred mares. The low incidence may be attributable to the diffuse type of placentation and strong uterine contractions. The causes are similar to those in cattle: the most common infection associated with retention of placenta being *Streptococcus genitalium* or *zoepidemicus*. The infection gets into the uterus after foaling due to wind sucking in the vagina or sudden rise of mare after the act of foaling. Retention may occur in the mare after abortion or prolonged gestation also.

### Symptoms

Normally, the foetal membranes should be expelled within  $\frac{1}{2}$  to 3 hours after foaling. Before expulsion of placenta the mare exhibits colicky pains and lies prostrate. Some mares may not exhibit abdominal pain and appear normal and may even suckle their foals. In some delayed expulsion leads to colicky pains. The retained membranes are observed hanging from the vulva and in nervous mares it may be necessary to cut off and the cut end secured by string so that it may not be drawn in to avoid dangling sensation and kicking in the air. The separation is not quick in the non gravid horn and the remnant of placenta in the apex may serve as a focus of infection setting up severe metritis and laminitis. It is therefore customary to spread the retained placenta after it has been dropped to ascertain if the entire placenta has been expelled. Retention may be accompanied by uterine invagination, severe metritis with placenta floating in the uterus filled with foetid fluid.

### Prognosis

The prognosis is usually fairly good.

### Treatment

If the pulse and temperature of the mare is normal no treatment for retained placenta is indicated until about 24 hours. 3 to 7 ml of pituitrin or 40 to 100 I U of oxytocin may give favourable results during this period. The placenta may drop down within 24 hours. In such cases parenteral and intra uterine antibiotics are indicated. However if the placenta is still retained after 24 hours manual removal is advised. The mare is properly restrained, tail is bandaged, hind quarters and perineum are cleaned with soap and warm water. The operator wears protective clothing and after lubrication holds the hanging part of placenta, twists the foetal membranes so as to form a rope. The fingers of the other hand are passed along this rope and placenta is separated from the endometrium. At the same time traction is applied. Complete separation of villi is possible and entire placenta can be removed. Douching of uterus after the removal is not advisable but intra uterine antibiotics for 5-7 days would overcome infection and hasten involution. It is advisable to give injections of tetanus antitoxin, antihistaminics 30-60 mg of stilboestrol and 3-6 mg of oestradiol or 1-3 mg of ergonovine to overcome laminitis.

### Retention of placenta in the ewe and doe

Since manual removal is not possible in ewes and does it is advisable to administer parenteral and intra uterine antibiotics after cleaning the external genitalia and perineal region. In case placenta is hanging gentle pulling with twisting may help.



pushing and screwing action. This can be coupled with traction by the other hand. Those in the cervical area of the uterus and vagina should be removed first. The operator should reach non-gravid horn and later gravid horn. It is difficult to reach the ovarian end. Once the hand is introduced for manual removal, it should not be taken out frequently in order to avoid injury to the uterus and ballooning of vagina, thereby spreading further infection. If it is not possible to remove the entire mass, the hanging portion should not be cut unless it is too heavy. Manual removal should not be undertaken in cows having high temperature and necrotic vagina which indicates septicaemic condition. After removal of placenta, no douching should be done but broad spectrum antibiotics or sulphanilamides may be used to overcome infection. The dosage will depend upon the type and potency of the product.

If it is not possible to remove the placenta easily, force should not be employed. It is better to remove it the next day. However, sulpha and antibiotic pessaries may be placed in the meanwhile. The layman's practice of tying the weight to the placenta or cutting it close to vulva are not desirable. The weight may cause invagination of uterus — (Deshpande *et al*, 1971) — (Fig. 18a). It is advisable to ascertain that there is no portion of placenta left, as it may get locked up setting metritis in consequence of which recovery of endometrium and uterine involution will be delayed.

## 2. HORMONAL TREATMENT

Hormones have been extensively used for treatment of retained placenta. Stilboestrol with antibiotics has been recommended. However, it is experienced that in buffaloes, oestrogen therapy for retained afterbirth is not advisable,

since administration of synthetic oestrogen results in sudden suppression of milk yield. In certain number, it may lead to complete cessation of mammary function. However, variable results have been claimed depending upon whether the administration is done immediately after parturition or after a day or two. Pituitrin and oxytocin may be used but their action is questionable after 24-48 hours.

Many antiseptics were used in the past to overcome bacterial infection e.g. charcoal, boric acid, acriflavin, bismuth subnitrate, bismuth formic iodide, bismuth thymol iodide, chlorine preparations, iodine in oil, silver oxide, BIPP, etc., but their effectiveness is undetermined.

Sulpha therapy has a limited use. Broad spectrum antibiotics are very effective. Intra-uterine penicillin, streptomycin, oxytetracycline (terramycin), chlortetracycline (aureomycin) and furacin are extensively used and proved beneficial. It is however essential to maintain the blood level at least for 5 days or even longer.

In case systematic symptoms appear, supportive treatment by way of saline and glucose, blood transfusion and parenteral antibiotics are indicated. Parenteral injections of 4-5 ml. methargin have proved beneficial.

## Complications

Retention of placenta may cause slight to severe lesions in component parts of genital tract. These include vaginitis, necrotic cervicitis, endometritis, metritis, perimetritis, parametritis, hydrosalpinx, pyosalpinx, salpingitis, oophoritis, bursal adhesions and placentitis leading to delayed uterine involution, drop in milk yield and infertility. Parturient tetanus, gas gangrene and foot rot are other possible complications.



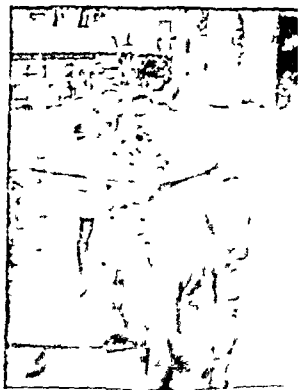
↑ Fig 18 a Invagination of uterine horn in a she buffalo

↓ Fig 18 b: Prolapse of uterus in a she goat



Fig 18 b Prolapse of uterus in a

Fig 18 c Abscess formation follow ventral vaginal rupture



### Retention of placenta in the sow

Retained placenta in sow is rare. The condition can be diagnosed after counting the expelled membranes and the actual number of piglets born. The affected sow is restless, does not nurse the piglet and shows symptoms of metritis with purulent discharge containing placental shreds. It is advisable in such cases to inject 20-30 units of oxytocin or pituitrin or stilboestrol together with parenteral and intra-uterine broad spectrum antibiotics. Usually the placenta is retained in the apex of the uterine cornua and in certain cases, it may be necessary to undertake laparotomy or even hysterotomy for its removal (Arthur, 1975).

### Retained placenta in the bitch and cat

Retention of placenta in bitch and cat is rare but may be encountered in small toy breeds. The condition is suspected when the animal voids dark green foetid vaginal discharge 12 hours after parturition. The discharge is brownish in cat. Retention may be confirmed by a radiograph in valuable pets. The vaginal exploration may reveal shreds of placenta which can be wound on the tip of finger or a padded forceps and removed if possible, failing which laparotomy is indicated. Intra-uterine antibiotics, pituitrin or pitocin 10 to 20 units and supportive treatment are suggested. If placenta is not expelled within 24 hours, it may lead to acute septic metritis when hysterectomy should be performed. If not attended to early, uterine wall may get necrosed leading to death.

## II. POST PARTUM HAEMORRHAGE

Post partum haemorrhage is the bleeding from the uterus and may be due to trauma or lacerations or rupture of the genital organs. It may follow abortion,

rupture of vessels during removal of foetal placenta, rapid expulsion of placenta, accidental instrumental injuries or hands of obstetrician during caesarian or foetotomy resulting in tear or cut in maternal caruncles and its stalk in cow and ewe, lacerations or rupture due to foetal extremities, forced traction on foetus, uterine atony in uterine prolapse, rupture of vessels in broad ligaments associated with aneurysm or degenerative changes in the arteries especially in mare. In bitches McMullen *et al*, (1964) reported invagination of the portion of the horn responsible for haemorrhagic discharge from the vagina.

Haemorrhage can also occur from broken ends of umbilical cord.

### Symptoms

Symptoms depend upon the degree of bleeding. Mild bleeding is of no consequence but severe bleeding is indicated by general symptoms such as quick, weak pulse, throbbing of heart, pale mucus membranes, unsteady gait, prostration, cold extremities, sweating, convulsions, shock and death.

In some cases, the animal shows abdominal pains, pawing and anxious look at the flanks. Haemorrhage at the vulva is obvious but diagnosis is difficult in internal haemorrhage.

### Treatment

The primary aim of the treatment is to locate the source of bleeding and if possible, ligate the blood vessel in order to control bleeding. Mild cases require no treatment, but in severe cases, 20-50 units of pituitrin controls haemorrhage by contracting uterus and its vessels. Cold packs on the loins are advised. A towel or a bedsheet dipped in water, per chloride of iron, adrenaline or any other styptic may be introduced in the vagina or uterus. Clots, if any,

may be removed after 24 hours and paravaginal use of methargin, coagulin, stadtren are useful in controlling the haemorrhage. A sedative dose like squalid is advisable in mare. Supporting therapy by way of glucose saline or blood transfusion is recommended to overcome the lost fluids and to maintain electrolytic balance. In case of parasitic aneurysms, rapid laparotomy and suturing of the vessel is indicated.

### III. TRAUMATIC LESIONS OF GENITALIA DURING AND AFTER PARTURITION

The traumatic lesions of genitalia include:—

- (i) Haematoma
- (ii) Prolapse of uterus (Fig. 18b)
- (iii) Prolapse of perivaginal fat
- (iv) Recto-vaginal fistula
- (v) Rupture of uterus
- (vi) Rupture of vagina (Fig. 18c)
- (vii) Rupture of perineum
- (viii) Rupture of intestine
- (ix) Rupture of bladder
- (x) Rupture of sacrosciatic ligaments.

These conditions have been dealt with in chapter on 'Surgical Interventions'.

### IV. METABOLIC DISEASES

#### 1. Milk fever (*Parturient Paresis*, *Hypocalcaemia*, *Puerperal apoplexy*)

With the advent of breeding cattle and buffaloes for enhanced milk production, the incidence of milk fever is on the increase.

Milk fever is essentially a metabolic disorder in cows and buffaloes occurring prior to, during parturition or within 72 hours after parturition. This is characterized by hypocalcaemia. According to Jonson (1960) the milk fever is a adaptation disease (man made) correlat-

ed to high milk yield. At the same time, neither all the high producing suffer from milk fever nor all the cows with low milk yield are prone to it. The other predisposing factors include age, breed and genetic, high milk yield and its persistency. Milk fever may recur in subsequent parturitions.

The work of Kronfeld and Ramberg (1970) and Nurmio (1968) has suggested that the present practice of giving high dietary calcium together with high calcium phosphate ratio to a non-lactating high pregnant cow stimulates the release of calcitonin or thyrocalcitonin — a substance which lowers blood calcium by inhibiting bone resorption — from the para follicular cells of thyroid which in turn inhibits bone resorption by parathormone. Thus, at the commencement of lactation, the demand for calcium is increased and ultimately the cow becomes hypocalcaemic and the milk fever ensues. Similar conditions can experimentally be produced by intravenous injection of large quantities of calcium (Albright and Blosser, 1957). The normal blood serum calcium level in a cow varies from 4 — 12 mg/100 ml. The milk is very rich in calcium and the onset of lactation means a very severe drain on calcium reserves. The total blood calcium is about 12 gm and this has to be removed every hour in a high producing cow. Even at parturition there is a decrease of 1 to 2 mg/100 ml of blood and hence a level of 8 — 9 mg/100 ml is considered as normal during parturition. Any value lower than this (4 — 7 mg/100 ml) will lead to milk fever (Jonson, 1960). Calcium ions are necessary for normal transmission of nerve impulses to the muscles. When the interstitial calcium content becomes low, such a transmission cannot occur resulting into paralysis of the skeletal muscles.

terised by hypoglycaemia, Ketonaemia and Ketonuria. Pehrson (1966) described ketosis of three types:

- (i) Primary spontaneous ketosis — hereditary
- (ii) primary nutritional
- (iii) Secondary ketosis due to metritis, abomasal displacements, traumatic gastritis and other diseases. Of these, the primary nutritional type is the commonest. Depending on the site of ketogenesis, Kronfeld and Emery (1970) described three types of Ketosis viz
  - (i) Alimentary (ii) Hepatic (iii) Mammary

This disease is essentially due to lack of carbohydrates. The blood glucose and ketone levels in a normal and acetonaemic animals are shown in table 12.

Table 12 showing blood glucose and ketone levels in normal and acetonaemic cows: —

Sr. No.	Item	Normal animals (mg. per 100ml.)	Acetonaemic animals (mg. per 100 ml.)
1.	Blood glucose	40-60	18-40
2.	Blood ketone	2-15	15-75

### Causes

The exact cause is not known. However, it may be related to the lack of carbohydrates in the nutrient, absorption of aseptic lochia in the uterus, feeding of too high concentrated diet and absorption of toxic products from the alimentary canal. The acetone and acetone bodies (3 hydroxy butyric acid) make their appearance in the blood, setting up acidosis and are excreted through lungs, urine and milk. In digestive type of disease which is of frequent occurrence the symptoms

include loss of appetite, constipated bowels, drop in milk yield, rapid loosening condition, staggering gait and inability to rise. In nervous type which is less frequent, the symptoms are more pronounced and are indicated by marked dullness. The other symptoms are shivering, nervousness and tendency to lick. Paresis, anorexia and drop in milk may also be observed. The most important symptom is the sweet, acetone like odour in the shed around the animal and in urine and milk.

In authors' experience, the buffaloes refuse to take greens and concentrates but they take only hay. There is sudden drop in milk yield.

### Diagnosis

Course of disease varies from few days to few weeks. The condition has to be differentiated from post partum metritis. However, diagnosis can be confirmed by detecting acetone bodies in urine or milk with Rothera's test or Ross reagent.

### Nitroprusside test (Rothera)

Saturate 20 ml of urine with ammonium sulphate by shaking with the crystals in a test tube. Add 2-3 drops of concentrated ammonium hydroxide and few drops of freshly prepared 5% solution of sodium nitroprusside and shake. A positive test is indicated by the development of permanganate tinge which gradually deepens. A brown colour is not a positive test.

### Prognosis

Prognosis is usually good. The mortality rate although very low, body weights and milk production are greatly decreased and hence this disease is of economic importance.

### Hypocalcemia in Cats

This is rare in the cats. However the condition has been reported by Michael (1960) and James Ashburner (1961). It is usually seen in queens nursing a large litter size.

In the affected cats, anxiety, nervousness increased abdominal breathing, slight fever, stiffness and intermittent tetanic muscular spasms are noticed. In advanced stages, vomiting and coma may ensue. The pupils are dilated and the cornea looks dry and temperature becomes subnormal. Prognosis is usually grave without treatment. Treatment consists of administration of 5 ml. of 10% calcium gluconate. In extremely nervous cases, a small dose of tranquilizer is beneficial.

The affected queens should not be allowed to continue nursing and the kittens should be hand fed 4 to 5 times a day. Calcium lactate or calcium gluconate 30-35 mg. morning and evening should be given to the affected queen for a period of two weeks.

### Symptoms

The disease most frequently sets in 12 to 72 hours after parturition. It is not however uncommon to observe the disease during parturition or even before parturition but never before the appearance of milk. In exceptional cases it may be observed in 6 to 7 weeks after calving or after abortion (Marathe, 1977). The longer the interval after parturition, the milder is the attack. The affected animal becomes uneasy, the appetite is lost and ruminations are suspended. The respiration is hurried and the pulse normal. Body temperature is subnormal, staggering, incoordination and stiffness in gait are noticed. The animal lies down on the floor either fully extended or resting on sternum with the head

turned towards the shoulder or flank. This is a characteristic position in milk fever with tonic spasms of the cervical muscles on one side resulting into 'S' curve in the neck. The urination and defecation are suspended, constipation and tympany may ensue. Mucous membrane of the mouth is pale and saliva and froth may dribble. The milk secretion is diminished or suspended. The pupils are dilated and light reflexes are lost. If the disease occurs during parturition, the act may be suspended. Coma develops and death may ensue with 6 to 24 hours, if untreated. Prognosis is usually good if treated promptly. Some cases may recover spontaneously.

### Differential Diagnosis

Milk fever has to be differentiated from metritis and postpartum paralysis.

### Treatment

The logical treatment in milk fever is to restore serum calcium to normal level and this can be achieved by intravenous injection of 700-1500 ml. of 20% calcium borogluconate. Beneficial results have been claimed by giving intravenous drip and half the quantity by subcutaneous route. Insufflation of air in the udder has not yielded encouraging results. Irradiated Ergosterol or large quantities of Vitamin D can also be useful but the value is questionable. Complete emptying of udder is not advisable for a couple of days. Drenching during this condition is contra-indicated as it may result in aspiration pneumonia. The feed should have a normal calcium — phosphorus ratio.

### 2. Ketosis (Acetonaemia)

This disease of dairy animals usually occurs 7 to 10 days and rarely between 10 — 60 days post-partum and is charac-

globinaemia, haemoglobinuria and anaemia occurring within 2 — 4 weeks after calving.

The affected cow shows lack of appetite, weakness, dehydration, icteric mucous membranes, increased pulse and respirations, constipation, in severe condition prostration and death in 3 to 5 days. The serum phosphate level in severe cases is as low as 0.5 to 1.5 mg/100 ml. of serum. Treatment consists of blood transfusion, intravenous injection of compounds containing phosphorus or monosodium phosphate and bone meal in feed orally. The disease should be differentiated from leptospirosis and pyelonephritis.

## VI. PARTURIENT LAMINITIS (PUERPERAL LAMINITIS)

It is the inflammation of sensitive laminae (laminitis) and is commonly observed in mare and less frequently in cow, ewe and sow.

### Aetiology

The exact cause is not known. The condition usually occurs within two to three days after parturition. Out of 170 cases of bovine laminitis, 24% occurred within this period (Nilsson, 1956). In most cases, laminitis is due to septic metritis, subsequent to retention of placenta. It may be due to disturbances in blood circulation or suppression of lochia. It may follow intestinal congestion or administration of drastic purgatives. The condition has been ascribed to momentary paralysis of the nerves supplying to keratogenous apparatus. However, the main cause appears to be infection.

### Symptoms

The symptoms are characteristic of inflammation of feet and develop within

24 hours after parturition. The hoof is hot and strong pulsation is noticed in the planter arteries. The animal loses appetite, pulse is rapid, respiration is hurried, one or both hind limbs are affected. The animal is in agony. Profuse sweating follows and milk secretion is reduced. The animal ignores the young one, the stance is typical — stands with hind legs under the body, thereby avoiding body weight on the affected limb. If unilateral, the affected limb is kept in raised position. The disease runs the course of 4 to 8 days; the affected animal usually recovers, but in rare instances, death may occur due to nervous exhaustion subsequent to pain.

### Prognosis

Usually good if treatment is resorted to in time

### Treatment

Treatment consists of application of cold packs to the hoof or cold water irrigation. Antihistaminics may be used as a supportive therapy. Metritis should be overcome by appropriate treatment with intrauterine antibiotics. Surgical treatment in the form of shoes for laminitis may be tried in severe cases so as to relieve weight on the frog.

## VII. PUERPERAL TETANUS

Puerperal tetanus may occur in the mare and cow and less frequently in other species after parturition. The condition usually follows uterine prolapse, retention of placenta, dystokia, puerperal metritis and traumatic injuries to the genital tract, usually within 1 to 4 weeks after parturition. The symptoms and treatment are similar to

## Treatment

Treatment consists of restoring normal blood glucose by injecting 500-1000 ml. of 40% glucose intravenously. After testing for acetone bodies, treatment may be repeated, if necessary. Sodium propionate — 100 — 300 gm. fed daily or 200 — 500 ml. of propylene glycol orally, 450 ml. of glycerol orally, especially in buffaloes has proved beneficial. Glucocorticoids intramuscularly, ACTH 20 — 100 I.U. intramuscularly, 10 — 30 gm. chloral hydrate daily and soda-bicarb may be administered to overcome acidosis. To avoid recurrence, adequate energy levels in diet should be fed.

### 3. Parturient eclampsia (parturient tetany)

This condition is characterized by tonic and clonic spasms or convulsions without loss of consciousness. It is seen in mare, cows, ewe, goat, bitch and sow usually after parturition. The exact cause of the disease is not known and no definite post-mortem lesions are observed. Cold climate is a predisposing factor and the condition may be due to retention of waste products in the system during pregnancy.

## Symptoms

Mare and cow become nervous, restless, twitching and clonic spasms of muscles set in, become very stiff and the animal is unable to rise. Respirations are hurried and mare sweats profusely. The urine is not discoloured as in azoturia. Asphyxia follows and animal may die within one or two days. In cows, parturient eclampsia is also reported and the symptoms are similar to those in parturient apoplexy. It is more common in primipara and those in poor condition. Clonic spasms of body and

limb muscles lasting for 2 to 3 hours is characteristic of disease.

Parturient eclampsia has also been reported in ewe and goats. The onset is followed by convulsions of all muscles, particularly the jaws, rolling of eyes and salivation. Animal is unable to rise and coma sets in.

The disease is observed in suckling bitches and is very common in miniature breeds occurring within 2 to 8 days postpartum. The affected animal becomes uneasy, anxious and the respirations are hurried. She is unable to rise and lies prostrate. The tonic spasms of muscles of body set in. Grinding of teeth, foamy mouth and trembling of jaw muscles may be observed. The defecation and urination are suspended and mammary glands are distended and hot. The disease may last for one to two days or attacks may recur at an hourly interval.

The disease is rare in cats.

Parturient eclampsia also occurs in sow 3 — 4 days after farrowing. Sow loses appetite, ignores the new born, lies prostrate, defecation and urination are suppressed and mammary secretion ceases. Spasmodic contraction of muscles are seen, respirations are hurried and there is loss of sensitivity. The disease may last for 24 to 36 hours.

## Treatment

Treatment consists of intravenous injections of calcium gluconate, narcotics and tranquillizers like chloral hydrate, sodium pentobarbitone or corticosteroid therapy.

## V. POSTPARTUM HAEMOGLOBINURIA, HYPOPHOSPHATAEMIA

This disease is not of common occurrence. Old dairy cows are usually affected. It is characterised by haemo-



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those in tetanus and consists of administration of tetanus antitoxin, parenteral and intra-uterine antibiotics and tranquillizers. Good nursing is a must. Recovery may ensue if attended to in time.

### VIII. POSTPARTUM PARALYSIS

Post partum paralysis is less common than antepartum paralysis and most commonly observed in mare, cow and other species. The most common forms of paralysis are gluteal, obturator and peroneal nerve paralysis which are brought about by pressure of foetus on gluteal nerve during hiplock dystokia and injury to peroneal nerves. Forced traction on the large foetus may also lead to paralysis. Depending on the severity, one or both limbs may be affected. The animal is unable to rise, if bilateral, but may stand with one limb raised, if unilateral. In obturator paralysis, the animal stands with limbs abducted. The course of the disease may last for weeks or months and the prognosis is usually good.

#### Diagnosis

The condition should be differentiated with the aid of previous history and also from milk fever, metritis, inflammation of spinal cord and meningitis, injuries or pelvic fractures.

#### Treatment

Good nursing is essential, abducted limbs may be tied by means of straps or ropes around fetlock or hocks. Massage of limbs, use of slings and nervine tonics may be useful.

Other puerperal infections viz. salpingitis, metritis, cervicitis and vaginitis have been described in details elsewhere.

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In the bitch and cat, manual palpation of the enlarged abdomen will give an indication of the number of foetuses in uterus. Continuous vomiting and thirst should be viewed with concern.

### Specific Examination

#### LARGE ANIMALS

Specific obstetrical examination should be carried out in clean and hygienic surroundings taking due precautions for the safety of the operator and the animal. Obstetrical instruments and appliances (List) be kept ready and the animal duly prepared prior to carrying out the actual examination. It is also essential to evacuate the rectum and bladder to facilitate manipulations and avoid complications. After thoroughly cleaning the external genitalia and perineum and after washing and lubricating the hands, the operator passes the hand in the vagina. During this examination he has to note, if there is any trauma to vagina, cervix or uterus possibly caused by malhandling by laymen.

Pathological conditions obstructing the vaginal passage should be noted. Examine the birth canal if it is dilated, moist, dry, oedematous, necrotic or twisted (torsion). If the foetal parts are not in the vaginal passage, the cervix should be examined for dilatation. Examine if the foetal membranes are intact or ruptured. Ascertain if the foetus is dead or alive. In case the foetus is decomposed hair can be pulled out easily. The presentation, position and posture of the foetus and teratological defects, should be determined.

The fore-limbs can be identified by the planter surface of the hoof facing downwards and presence of two joints — fetlock and knee — between hoof and elbow whereas in case of hindlimbs, the volar-surface of the hoof faces upwards and there is only one joint-fetlock

joint between hoof and hock. The joint movements are independent in the fore limbs, flexion occurring in the same direction while joint movements are interdependent in hind limb. In some cases repulsion of the foetus may be necessary in arriving at correct diagnosis.

Such an examination would lead to accurate diagnosis for deciding the further line of action for relieving dystokia.

List of Obstetrical instruments and appliances:

#### Instruments

1. Obstetrical set preferably Thygesons embryotomy set.
2. Two cords — 4-5 feet long,  $\frac{1}{2}$ - $\frac{3}{8}$  inch thick with loop at one end.
3. Nylon calving ropes.
4. Two blunt hooks—Large and small.
5. Two sharp hooks—Large and small.
6. Lunds anal hook.
7. Repellar
8. Guarded embryotomy knife.
9. Cutis separator.
10. Bone saw or chisel.
11. Obstetrical chains two.
12. Epidural set.
13. Krey-Schottlers hook.
14. Clinical thermometer.

#### Medicines

- (a) Stimulants: Coramine.
- (b) Sedatives: Chloral hydras, Chloroform.
- (c) Uterine stimulants: Pituitrin, Methargin, Calcium boro gluconate.
- (d) Antiseptics: Dettol, Savlon, Pot. permanganate.
- (e) Lubricants: Obstetric jelly, Common oil.
- (f) Injections: Xylocaine, Procaine Penicillin, Terramycin.

Others : Soap, Towel, Leather bag, protective clothing, Cotton, Tincture iodine.

# Chapter 8

## General Considerations in Obstetrical Practice

### Approach to an Obstetrical case

Every case of dystokia by itself constitutes a clinical problem and correct diagnosis of the condition is necessary in obstetrical practice. Prior to handling of dystokia cases, complete history of the animal should be obtained. History should include: (1) Duration of gestation to know if it is full term or premature (2) previous breeding records such as primipara or pluripara, any troubles during previous parturition including twins, monsters etc. (3) information about housing, feeding, management and exercise during pregnancy (4) any disease noticed during gestation period (5) Since when the symptoms of restlessness, anorexia have been noticed (6) onset and nature of straining—weak, intense, regular and duration (7) when the straining has ceased (8) time of appearance of water-bag, its rupture and type of foetal fluids (9) in multiparous animals enquire about the number of young ones born already and their condition — living or dead (10) nature of earlier attempts to relieve dystokia (11) note if any of the foetal parts have appeared at the vulva (12) ascertain if the animal is completely or partially off feed. Vomiting may be noticed in dogs and cats,

(13) note if there is tympany and whether the animal is exhausted (14) note the condition of mammae and observe if there is oedema.

Special attention should be paid to the duration of labour pains and stage of parturition. If there is delay of over 24 hours and cessation of labour pains, then it may indicate a dead foetus, exhausted uterus, total loss of foetal fluids and beginning of putrefactive changes.

### General examination of the animal

Before handling any obstetrical case, the general condition should be noted. Pulse, temperature and respiration should be recorded and any deviation thereof be considered with concern. Visible mucus membranes should be observed to get an idea of internal hæmorrhage or shock.

The condition of the exposed foetal parts will give a clue as to whether the foetus is alive or dead.

There is a possibility of obturator paralysis or parturient paresis in cases of prolonged recumbency which should be ruled out. The nature of the discharge should be carefully observed for putrefactive changes or injuries in the genitalia.

Table 13  
SEASONAL INCIDENCE OF DYSTOKIA IN BUFFALOES

Season	Maternal	Foetal	Total	Reference
Summer	—	—	3.42	Gudi and Deshpande (1975)
	1.20	8.10	9.30	Deshmukh and Kaikini (1975)
Monsoon	—	—	5.46	Gudi and Deshpande (1975)
	17.50	59.30	76.80	Deshmukh and Kaikini (1975)
Winter	—	—	3.01	Gudi and Deshpande (1975)
	6.90	7.00	13.90	Deshmukh and Kaikini (1975)

dangerous in the mare since the labour is tumultuous, the difficulty is increased due to powerful straining and the foal may not live beyond four hours due to peculiar type of placentation. The foal undergoes rapid rigor mortis, decomposition and emphysema sets in bloating its size. The long neck and limbs of the foetus are difficult to manipulate. Injuries of genitalia become septic endangering the life of the mare.

In the ewe, postural abnormalities are very common particularly when there are more than one foetuses. Developmental irregularities are also relatively common. Wallace (1949) reported that 15% dystokia in sheep

were due to incomplete dilatation of cervix-'Ringwomb'.

In sows, uterine inertia and pelvic injuries are relatively more common causes of dystokia. Two foetuses simultaneously presenting at the pelvic brim is another cause (Becze, 1976).

In bitches, dystokia is quite common due to large size of foetuses. Incidence is highest in small (toy) breeds. However, dystokia is less common in dogs bred under natural conditions. Freak (1962) in his studies on canine dystokia recorded an incidence of 48.16% maternal and 51.84 foetal types (Table 14).

Table 14  
DYSTOKIA IN CANINES (FREAK, 1962)

Type	Condition	No.	%
A. Foetal Dystokia (Obstructive)	Relative oversize—one or more pups	77	
	Absolute oversize	15	
	Monstrosity or gross abnormality	2	
	Malpresentations without posterior presentation	12	
	Posterior presentation of first pup.	35	
Total		141	51.84%

# Chapter 9

## Maternal Dystokia

The term dystokia is used to indicate various types of obstructions in the process of parturition. Dystokia occurs when parturition in general and the first and second stages in particular are prolonged and when artificial aid becomes necessary for extraction of the foetus. The word is derived from Greek "Dys" difficult and "tokos" birth as against Eutokia normal birth. Dystokia depends upon the degrees of resistance as opposed to expelling powers. This resistance may be due to two factors:

- (a) *Maternal dystokia* due to abnormal conditions of the genital passage of the dam.
- (b) *Foetal dystokia* due to mal-presentations or abnormal conditions of the foetus.

The severity and frequency of these conditions vary in all the domestic animals. It is more common in the bovine 3.3 per cent (Williams, 1943). The incidence is much higher in dairy breeds than in beef and draught. It is more frequent in primipara than in pluripara and in those animals having previous history of pathological conditions in any part of the genitalia.

Leech *et al*, (1960) estimated that the national incidence of dystokia in

Great Britain was 1.78 per cent. Linde (1963) observed the incidence of dystokia varying between 6.8 per cent in pure bred SRB cattle to 15.6 per cent when they were crossed with Charolais in Sweden. Bane (1964) showed variation in the incidence of dystokia in SRB heifers depending on the breed of calf's sire. Grommers *et al*, (1965) recorded 10.8% dystokia out of 1,391 calvings in Holstein-friesians. Ekes Bo (1966) observed the incidence of 10.3% out of 4,616 calvings in Swedish breeds of cattle.

Deshmukh (1975) in his studies on problems of parturition in buffaloes recorded a higher incidence of foetal dystokia (74.40%) than maternal dystokia (25.60%).

Gudi and Deshpande (1975) reported an overall incidence of 4% dystokia in buffaloes of which 1.7% were maternal and 2.3% of foetal origin.

The season wise incidence of dystokia in buffaloes is presented in Table 13.

The high incidence of dystokia observed during monsoon season may be explained on account of the fact that more calvings occur in that particular season.

Dystokia in mares is less common- 1.1% (Von Ottingen, 1921). Dystokia is

consists of inducing abortion or forced traction failing which embryotomy or caesarean may be resorted to.

## 2. Failure of cervix to dilate

Failure of cervix to dilate completely is a common cause of dystokia in cattle and buffaloes. Gudi and Deshpande (1975) recorded an overall incidence of this condition as 0.8% and 46.6% of the dystokia cases studied. This condition may occur both in heifers as well as in pluripara. In the latter group the condition is due to fibrosis of cervix, subsequent to injury during previous parturition. It may also be due to hormonal imbalance.

The animal shows weak labour pains transient in nature. The animal strains continuously leading to exhaustion. Vaginal examination reveals that the cervix is not properly relaxed. Forced traction in such cases is bound to cause cervical tear. Foetal limbs are occasionally felt in the fornix.

In such conditions however, there will be no further dilation even after several hours. In abortions too, the cervix fails to dilate properly and the foetus is retained which subsequently undergoes maceration. Incomplete dilation of cervix may rarely be encountered in uterine torsion.

Various methods have been tried to dilate the cervix such as hot and cold water packs on the loins, warm water enema, sterile warm water vaginal irrigation or mechanical dilation with digital manipulation or instruments. However efficacy of such treatment is doubtful. Hormone therapy (estrogens) in such cases may be of some use.

Incomplete dilation of cervix (Ring womb) is a major cause of dystokia in ewes — 15% (Wallace, 1949).

## 3. Vaginal Cystocoele or Hernia of bladder into Vagina

This condition is occasionally encountered in cow, buffalo and mare during parturition when the urinary bladder lies in vagina or vulva. There are two types:

### 1. EVERSION OF BLADDER THROUGH THE URETHRA

This frequently occurs in mare as a result of great dilatability of urethra and the vigorous straining efforts. The everted organ occupies the vulva and is seen inbetween the vulval lips as a red mass with two openings of ureters and meatus urinarius cannot be located. The bladder is empty with a corrugated mucosa. There is continuous dribbling of urine.

### 2. PROLAPSE OF BLADDER

This takes place through rupture of vaginal floor and the bladder lies in the vagina with its serous coat outermost. It increases in size gradually and meatus urinarius can be palpated. It is a smooth fluctuating mass.

Treatment consists of overcoming the straining by way of epidural anaesthesia with or without narcosis. This is followed by retropulsion of foetal parts occupying the vaginal passage. In case of eversion, it is necessary to invert the organ by manipulation and in case of prolapse to replace the organ in the pelvic cavity and to suture the ruptured vaginal floor.

## 4. Uterine torsion

### SYNONYMS

Uterine torsion, contorsio uteri, uterine volvulus, twisting of uterus on its long axis, revolution of uterus, torsio uteri.

Table 14 (Contd.)

Type	Condition	No.	%
B. Maternal dystokia (Obstructive)	Abnormality of maternal soft structures	4	
	Pelvic abnormalities (accidental)	1	
	Slackness of abdominal walls	3	
	Total	8	—
C. Inertia	Primary inertia	41	
	Secondary inertia	44	
	Nervous involuntary inhibition of labour	17	
	Slow initiation of labour		
	(Hormonal origin)	1	
	" " (Subclinical eclampsia)	7	
	Abortion near term	2	
	Death of foetus prior to whelping	10	
	Co-incident illness	1	
	Total	131	48.16%
Grand Total		272	

In cats, the incidence of dystokia is comparatively low.

### MATERNAL DYSTOKIA

Causes of maternal dystokia are as under:

#### 1. Pelvic Abnormalities

Dystokia due to pelvic abnormalities or injuries to pelvic bones is rare. Any general or partial change in the shape of pelvis is serious. Pelvic abnormalities may be due to rachitis, depression of sacrum, exostoses, fractures of hip bones, callus formation (Fig. 19), tumours, dislocations of hip joint, small pelvis for want of full physical developments and projection of anterior portion of pubis.

Deshmukh (1975) recorded an incidence of 1.20% cases of dystokia due to pelvic deformity in buffaloes.

Kunthawar and Kaikini (1968) recorded a rare case of maternal dystokia due to pelvic constriction in a horned goat.

Prognosis in such cases is usually grave and such animals should not be re-bred. Approach to relieve such cases

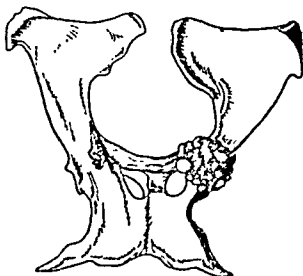


Fig. 19. Abnormality of pelvis due to callus formation.



axis The ovarian end is a narrow base on which it rests whereas the body of uterus remains fixed and therefore the nongravid cornua which is nonfunctional gets twisted along with its ligaments on account of instability of uterus and consequent torsion follows this being explained as a gravitational accident (Chauveau 1891)

According to Goubaux the cornua increase in length but attachment of broad ligaments to inner flanks of ilium remains fixed Projection of cornua one to two feet or so beyond the broad ligaments leads to torsion Combination of these two explanations may be considered for understanding the mechanism of torsion

#### (b) Functional anatomy

The manner in which the cow lies down and gets up is peculiar She goes down on knees first while sitting and elevates her hindquarters first before getting up Every time she does so the gravid cornua is suspended in the abdominal cavity for some time A slight fall or slip during this time or even a butt by a neighbouring cow may predispose to torsion

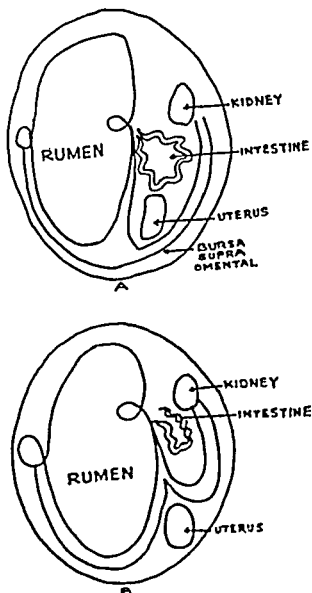
#### (c) Attachment of amnion

Partial attachment of amniotic sac to the allantochorion in bovines predisposes to torsion of uterus as a result of violent foetal movements The foetus in lateral recumbent position in early pregnancy while changing to dorsal one in later stages may drag the uterus along with its violent movements leading to torsion In solipeds the foetus is more or less free from uterine lumen and therefore torsion is rare

#### (d) Relation of pregnant cornua to bursa supracornualis

Saunders (1916) opined that when uterus lies inside the bursa supracornualis

there was good fixation This fixation is lost with advanced pregnancy when the uterus is outside the bursa This creates more space for the free movement of the organ and predisposes to the uterine torsion Normally, the nongravid cornua lies in the bursa (Fig 20)



**Definition:**

Torsion of uterus is defined as twisting or revolution of uterus on its long axis. It is common in cow and buffalo, occasionally in ewe and goats but rare in mare, bitch and sow. About 90% of torsions in cattle are observed only at parturition. This condition is of great scientific and economic importance in cattle.

**HISTORY**

Uterine torsion was first described by Boutrollac in 1766 and later by Lacoq (1837). Richner (1840) advised rolling of body in uterine torsion. Uterine torsion has since then been recorded by several veterinarians.

**INCIDENCE**

In most of the countries, uterine torsion is common in dairy cattle, the incidence and relative frequency varying from country to country, breed to breed and depends largely upon the animal husbandry practices and rearing conditions in vogue. The incidence was as high as 9.4% (201 in 2132 dystokia cases) reported by Johnik (1920). 20.25% dystokia cases in Switzerland were due to uterine torsion (Rohrbrasser, 1963). According to Lagerlof (1957), the incidence was high in Northern Sweden probably on account of hilly tract. Van Derkaay (1950) reported incidence of uterine torsion, as 10% in Holland (229 cases out of 2279 dystokias) during 1936-40.

Bach reported 183 cases of uterine torsion of which 15 had to be slaughtered and only 67% calves were viable, whereas Wyssmann had 38 cases in 100 dystokias (38%). Levens reported 103 out of 910 cases (11.3%). According to Johnik (1950), out of 2075 dystokia cases attended by him, 197 (9.5%) were due to uterine torsion. May (1950)

found that 30% of total dystokia cases attended in German mountainous areas were due to torsion. Grabherr (1949) attended 594 dystokia cases and found torsion in 200 (33%).

Uterine torsion was observed in 7.67% out of 1555 cases at New York state Veterinary college during 1945-53, with 11% mortality. The figures from the same institution for the period 1925-45 indicate 225 out of 1703 cases (13.2%) with 18.1% mortality of dams.

Van Egman and Matthijsen (1962) recorded a case of uterine torsion of nongravid horn. This was associated with hydrallantois, whereas Williams (1943) reported a case in which nongravid horn with torsion contained pus.

The incidence of uterine torsion in Surti buffaloes is relatively high. It was 38.7% (1300 cases out of 3357 dystokias) attended by Amul veterinarians during 1963-69. In only one case uterine torsion was associated with twin pregnancy. The figures from Aarey Milk Colony indicate uterine torsion to the tune of 7.8%.

**CAUSES**

Various causes have been put forth to explain the occurrence of uterine torsion and they are grouped as under.

**I. PREDISPOSING CAUSES****(a) Anatomical**

In cow, the cornua are curved, the greater curvature being dorsal lies free in the abdominal cavity and is supported by rumen, viscera and abdominal tunic. The broad ligaments are attached dorso-laterally to the ventral curvature and uterus is suspended by these ligaments. The uterus is in the form of an arc or U shaped loop with vagina and ovary at either ends of the arc. Twisting involves rotation of this arc on its transverse

## Nature of Uterine Torsion

### (a) STAGE OF PREGNANCY AT WHICH TORSION OCCURS

Uterine torsion is usually observed in advanced gestation period and is commonest during later part of 1st stage or early part of 2nd stage of labour (Roberts, 1956, Grabherr, 1949). Occurrence of torsion in early pregnancies is however not uncommon (Johnik, 1950). Out of 239 cases in surti buffaloes, 186 were observed at term, 29 between 9th and 10th month, 12 in 8th and 9th, 9 in 7th and 8th and 3 in 5th and 7th month of pregnancy (Chothani, 1972).

### (b) DIRECTION OF TORSION

The torsion may occur in two opposite directions i.e. when upper surface becomes left lateral or right lateral constituting left or right torsion. In other words the torsion may be clockwise or anticlockwise. Richter and Gotze (1960) recorded 60 to 70% right torsions. The high incidence of right torsion might be due to presence of rumen on left side. If the right horn is gravid right torsion may occur and vice versa. Grabherr (1949) recorded 62% right torsions. Of the 239 cases in surti buffaloes 231 torsions were on right and only 8 on left side (Chothani, 1972).

### (c) DEGREE OF TORSION

Torsion involves uterus, body, cervix, vagina and vulva. The cervix and vagina becomes twisted in a cord like manner followed by stricture of os and delivery through os becomes impossible. Torsion may be complete or incomplete. Incomplete torsion is most common. Although it is difficult to ascertain correctly with mathematical exactness the degree of torsion, it may be classified in 4 degrees depending upon the degree of rotation of uterus.

1st degree — Quarter torsion ( $\frac{1}{4}$ ) involving about  $90^\circ$ .

2nd degree — Half torsion ( $\frac{1}{2}$ ) involving about  $180^\circ$ .

3rd degree — Three quarter ( $\frac{3}{4}$ ) involving about  $270^\circ$ .

4th degree — Complete torsion (1) involving about  $360^\circ$ .

When the organ makes two complete turns the result is double torsion. Double and multiple twists are rare.

In cows,  $90^\circ$  and  $180^\circ$  torsions are more common than  $270^\circ$  and  $360^\circ$ . Berch (1903) observed only 2 torsions of  $360^\circ$  in 183 torsion uteri cases whereas Humer found only one of  $360^\circ$  in 700 cases studied. In Hanover clinic, out of 119 cases of torsion 74 were between  $90^\circ$  to  $180^\circ$ , 59 of  $270^\circ$  and 16 of  $360^\circ$ . Grabherr (1949) recorded 51% of  $90^\circ$ , 13% of  $180^\circ$  and 1% of  $270^\circ$ . In surti buffaloes of the 239 cases 59 were of  $90^\circ$ , 48 of  $180^\circ$ , 5% of  $270^\circ$  and 77 of  $360^\circ$  (Chothani, 1972).

### (d) SITE OF TORSION

The twist occurs usually in front of pelvic brim. The twist may be found at the posterior end of external os (post cervical) or on the body of uterus in front of os uteri internum (pre cervical), the former being more common. Johnik (1950) observed only 3 cases of pre cervical torsion out of 197 cases studied whereas in surti buffaloes out of 239 cases only 6 were precervical. Vander Kaay (1949) observed one in 299 cases (Fig. 21a).

than Murrah or Mehsana and Berari (Chothani 1972; Kaikini, 1977).

#### (f) Disease

Animals that have suffered from foot and mouth disease are prone to have torsion. The foot lesions affect the way in which animals lie down or get up (Grabherr, 1949).

### II. ENVIRONMENTAL

#### (a) Narrow platforms

Narrow standing space restricts the movement of animals. In case, the standing space is narrow, the animal may have to remain on knees for long time before getting up. They may stand in urine pit or in manger. They may roll from side to side while lying down. All these conditions predispose to uterine torsion.

#### (b) Hilly pasture

The incidence of torsion is high in pastoral countries where the region is hilly, broken or intersected and therefore seen more in animals who are on pasture rather than those who are stabled. Tapken cited by Williams (1943), observed it to the tune of 2.7% when animals were at pasture.

#### (c) Confinement with lack of exercise

Confinement favours uterine torsion. It was observed in 8.6% cases when animals were stabled (Tapken cited by Williams, 1943). The incidence is very high in buffaloes reared under stabled conditions in Bombay.

#### (d) Wallowing habits

Amul veterinarians have observed in Surti buffaloes, that advanced pregnant animals when taken for grazing or watering, roll in the mud or pond and also stand on knees while drinking water. The ponds may have slopy floors.

Slip or fall during wallowing may predispose to uterine torsion.

#### (e) Malnutrition

Malnutrition may result in weakness of ligaments and lack of uterine tone (uterine atony). According to May (1950) the uterine tone decreases during later stages of pregnancy and therefore predisposes to torsion.

### III. EXCITING CAUSES

#### (a) Foetal movements

Sudden violent foetal movements due to sudden jerks or blows or after intake of cold water may lead to torsion. These spontaneous, energetic, irregular movements carry the uterine cornua along with foetus leading to torsion. Malposition may assist in occurrence of torsion.

#### (b) External injury

Slips or falls on hind quarters, horn thrust (butting) by neighbouring cow in the flanks, rolling, struggling during casting, fall while jumping over a ditch or hedge, slip while descending a hill, tympany, severe toil, deformity etc. are some of the important causes of torsion.

#### (c) Irregular movements of pregnant animal

Violent and irregular movement of dam during pregnancy may cause torsion. These movements are more common in primipara than pluripara. The primipara becomes relatively more anxious and lies down and gets up more frequently, throws herself on one side or even rolls. Out of 239 torsio cases in surti buffaloes, 100 were in primipara (41%), 48 in 2nd lactation (20%), 50 in the 3rd (20%), 16 in the 4th (6%), 14 in the 5th (5%) and 11 in 6th lactation (4%) (Chothani, 1972).

ion is evident from the amount of tension on broad ligaments and arteries. It is difficult to palpate the uterus. Position of the foetus in utero in advanced pregnancy may indicate the degree of torsion. In majority of the cases of uterine torsion, the anterior portion of vagina is involved, the vaginal walls are slightly twisted resulting in stenosis. In left torsion, the spiral folds are directed forward and downward from the dorsum of vagina to the left (anti-clockwise). In right torsion the folds spiral downward and forward to the right side (clockwise). The dorsal vulval commissure may be pulled to the left or right side which can be markedly observed. Tumefaction of vulva is not marked and it remains dry.

In severe cases of torsion in late pregnancy, the blood supply is greatly interfered with due to pressure. The animal may show complete anorexia and constipation or foetid diarrhoea, suspended ruminations and ruminal activity, very rapid and weak pulse, quick respirations, expiratory grunt, normal to subnormal body temperature, cold extremities and difficult micturition due to involvement of bladder in the twist. Shock, collapse and death may ensue within 24-72 hours. The foetus may die, become emphysematous and macerate or may become mummified as a static foetal cadaver (Williams, 1921). The rectal examination reveals adhesions, parametritis or even an extra uterine foetus in cases of uterine rupture.

#### Symptoms of torsion at the time of parturition

The symptoms at this time are very mild. The animal becomes uneasy, restless may show colicky symptoms and slight anorexia. The abdominal straining of second stage of parturition is

either absent or mild and intermittent as the entrance of foetus in pelvis is obstructed by the twist. In spite of the history of initiation of labour for over 8-18 hours, no progress in the process of parturition is observed.

Respirations are increased and heart rate is elevated to 80-100 per minute. In cases with complications of uterine gangrene, rupture of uterus, emphysema and other toxic conditions, rapid and weak pulse, subnormal body temperature, cold extremities and possibly foetid diarrhoea may be present. The dorsal vulval commissure is pulled to one side either to the left or right.

If the twist is of  $90^\circ$  —  $180^\circ$ , discharge from the vagina is noticed. In such cases, waterbag may come out if there is cervical dilatation and the limbs of the foetus may be pushed into the vagina due to severe straining. In torsions involving  $360^\circ$  or more, the vaginal passage becomes narrower and the cervix cannot be palpated.

#### Diagnosis

Diagnosis is based on the history furnished by the owner and symptoms exhibited by the animal. The diagnosis of left or right torsion is possible by pervaginal examination wherein cork-screw like rugae or mucus folds would be palpated which indicates the direction of twist. The number of rugae are suggestive of degree of torsion. The vulval lips are drawn in and look buried. The foetal membranes are intact in  $180^\circ$  torsion and

account of different anatomical positions of uterus and ligaments.

### Symptoms

The symptoms of uterine torsion during the gestation period in the bovine may be completely lacking if the torsion is of a mild degree ( $45^\circ$  or  $90^\circ$ ). If the torsion is  $180^\circ$  or more, symptoms of abdominal pain, anorexia, constipation, lack of rumination, weak and slow rumen contractions, rapid pulse rate, restlessness or colicky symptoms and treading or tail switchings may be expressed. These symptoms should not be confused with traumatic gastritis, indigestion, pyelo-nephritis or intussusception. A

pregnant animal over 6 months if showing above symptoms should be subjected to rectal examination.

In the right torsion, the right broad ligament is pulled downwards under the twisted uterine body and vagina whereas the left one is tightly pulled across and over the dorsum of cervix, corpus uteri and vagina towards the right side. In left or anti-clockwise torsion, the right broad ligament crosses over the dorsum of twisted portion to the left side and the left one is pulled tightly under the twist (Fig. 21b). The middle uterine arteries are slightly stretched on both sides. The severity of tor-

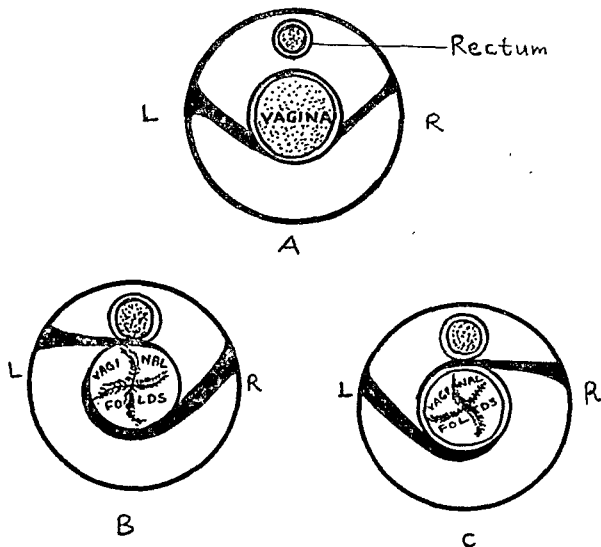


Fig. 21 b. Diagrammatic sketch of normal position of uterus, broad ligaments and vagina (A)  $180^\circ$  right torsion of uterus (B),  $180^\circ$  Left torsion of uterus (C).

### (b) *Use of instruments*

When correction by hand per vaginam is not possible, the use of detorsion rod as described by DeMott and Roberts (1945) or Derreau's uterine retroverser or Camerer's torsion fork may aid in the detorsion. The latter is being extensively used. It consists of a 75 cm central rod with pronged fork on one end and a wooden cross handle at the other. The canvas cuffs are tied to the forelimbs of the foetus if accessible and the prongs of the fork are inserted in the cuffs as high as possible. The detorsion is effected by rotating the crossbar in the desired direction.

### 3 ABDOMINAL BALLOTMENT

Benesch and Wright (1951) recommended ballotment as an aid to manual correction of torsion through the birth canal or in rolling the cow. In the left torsion, assistant on the left side pushes downward and inward in the upper left flank region whereas the assistant on the right side pushes upward and inward in the lower right flank region. In right torsion the upper right flank and lower left flank are pushed very strongly by closed fists. The assistants push alternately at regular intervals in quick succession which causes the uterus to roll from side to side and the resultant swing is of gr

### 4 ABDOMINAL TAXIS, LAPAROTOMY FOR INTRA ABDOMINAL CORRECTION

The operation can be done in standing position 6" — 8" long incision in the right para lumbar fossa is taken and uterus exposed. In case of right torsion the hand is passed below the uterus and above the uterus in case of left torsion. The foetal parts are seized and it is rocked up and down and turned to the opposite side of torsion. However presence of adhesions, heavy weight of uterus, its smooth slippery surfaces and limited space interfere with the operation.

### 5 CESARIAN

If much time has been already lost it is worthwhile to relieve the foetus by cesarian section. In cases of incomplete dilatation of cervix and dead emphysematous foetus or uterine rupture, cesarian is of great value.

The general condition of cow and birth canal and the uterus should be examined carefully. Peritonitis, internal bleeding due to rupture of large vessels, retained placenta, septic metritis are the common sequelae to torsion uteri and require appropriate palliative treatment.

## Prognosis

Prognosis of torsio uteri occurring prior to parturition depends upon the degree of torsion, severity of symptoms and its duration. Prognosis is fair to good in mild cases. It is good in early diagnosed cases of  $180^\circ$  to  $270^\circ$ . In severe and neglected cases, prognosis is grave.

## Treatment

Detortion of the uterus is of prime importance and can be effected by one of the following methods:

1. Rolling of the dam.
2. Rotation of foetus
3. Abdominal ballotment
4. Laparotomy
5. Cesarean section

### 1. ROLLING OF THE DAM

This method is the simplest one for relieving uterine torsion. Rolling may be done on a slope. Sedatives such as chloral hydras, largactil or sequil may be administered in excitable animals. The udder should be emptied to avoid possible injury. The front and hind limbs are secured separately. The object of rolling is to suddenly and rapidly rotate the cow's body in the same direction as that of the twist. The uterus remains stationary during rolling.

After ascertaining the direction of torsion accurately the cow is laid down in lateral recumbent position on the same side as the direction of torsion. The cow should be rolled in the same direction as that of torsion in rapid successions and coordinate pulling on the ropes tied to the fore and hind limbs. After the cow has been rolled through  $180^\circ$ , her body must be pushed slowly over the legs and sternum so as to continue the rolling in the same direction. It is advisable to keep the

hand in vagina or to grasp the foetal parts while the rotation is being effected. It is very necessary to examine the vaginal passage to find out if the rolling is effective. This can be judged by the relaxation or tightening of the vaginal mucous folds. If the rolling has been effective, the cervix becomes dilated and foetus can be palpated. On successful termination of the rolling, foetal fluids gush out of the uterus. If the vaginal spiral folds get tightened it indicates that the rolling has been done in wrong direction. In such cases the direction of rolling should be reversed.

Rolling can also be done on the spot. The animal is placed on the back, the limbs are tied and the animal is rocked from side to side.

### 2. ROTATION OF FOETUS

#### (a) *Direct detorsion of foetus per vaginum or vaginal taxis*

In this method, the foetus and uterus are rotated through the birth canal. Majority of the torsions are usually diagnosed at the time of parturition when the cervix is relaxed and dilated and since most of the torsions are less than  $360^\circ$ , the foetus can be grasped and rotated by the hands passed through the twisted vagina. Hind quarters should be kept in an elevated position. Straining may be controlled by epidural anaesthesia and the passage lubricated, if dry. After the correct diagnosis, hand is introduced deeply as far as possible. The foetal limbs or head is grasped and jerked swiftly in the opposite direction. Care should be taken to avoid rupture of waterbag since the foetus enclosed in waterbag gives a better hold for swing movement. Benesch and Wright (1951) advised initiation of foetal reflexes by pressing on eyeballs of foetus, which assists in correction of early cases of torsion.



animal tries to suppress abdominal contractions leading to uterine inertia. Transport of high pregnant animals over long distances is also a cause for inertia, possibly on account of stress.

### SYMPTOMS

The animal shows all signs of parturition such as mammary activity, pelvic changes, dilatation of cervix and presence of foetus in its membranes at the pelvic inlet. The animal may show few abdominal contractions but there is no progress in the process of foetal expulsion.

### TREATMENT

Immediate treatment should be rendered when it is known that second stage of labour has commenced but without any progress. In large animals, rupture of membranes, correction of malpresentation, if any, sufficient lubrication and gentle traction of the foetus often proves useful. In certain cases, intravenous injection of 500 ml. of calcium borogluconate (25%) gives good results. If the cervix is properly dilated and no postural abnormality exists, injections of posterior pituitary extract or 2 — 10 ml. oxytocin (20 — 100 I.U.) in large animals and 1 — 2 ml. (10 — 20 I.U.) in small animals may be administered to stimulate uterine contractions. On failure to release the foetus within 3 to 4 hours by above means, cesarian is indicated in bitches.

### SECONDARY UTERINE INERTIA

Secondary uterine inertia occurs subsequent to difficult labour due to exhaustion of uterine musculature and is also known as inertia of exhaustion. Dystokia is the primary factor in initiating secondary uterine inertia.

Secondary uterine inertia occurs in all species and can be prevented if detected earlier. Malpresentation and

mal-positions of foetus, foetal oversize, excessive amniotic and allantoic fluids, monstrosities, twins, malformations of foetus and narrow birth canal are the causes of secondary inertia. The animal shows no progress in parturition after the second stage. In such cases the membranes are ruptured, cervix is dilated and the birth canal appears dry.

If dystokia is prolonged, the uterus is often tightly contracted around the foetus. The uterine muscles anterior to cervix contract tightly in the form of a ring and this resembles the retraction ring, Bandle's or contraction ring dystokia in the human.

The diagnosis is based on history, and symptoms which can be confirmed by intra-uterine examination. In multiparous animals, cessation of labour pains after the birth of one or two young ones is a diagnostic sign of secondary uterine inertia. The prognosis is usually guarded. Retained placenta, metritis, delayed involution and pyometra are the common complications.

The essential aspect of treatment consists of relieving the dystokia after appropriate correction, sufficient lubrication and gentle traction on the foetus. In the presence of strong contraction rings which reduce the lumen of uterus, embryotomy or cesarian may be resorted to. In multiparous animals approach to such a case depends upon the duration of the labour, number of young ones still unborn, their condition and degree of uterine infection. In sow, bitch and cat relieving of the young one causing dystokia often restores the normal process. But in prolonged cases, hand or forceps delivery per vaginam is indicated for the remaining young ones. Hysterotomy should be the choice of operation in cases beyond 12 hours duration since all foetuses in the cornua

month of gestation. Sand (1918) observed that torsion occurs mostly on the right side. Johnik (1951) found ten right torsio-uteri out of 16 cases studied by him. In one of the mares the right torsion repeated during the next gestation on the same side. Quarter torsion is more common.

The causes are similar to those in cow. Rolling during colic predisposes to uterine torsion. Symptoms are also similar to those in the cow but colic is severe. A pregnant mare with colic should be examined for the presence of torsio uteri. Colon may get obstructed and there is difficulty in passing faeces. Diagnosis can be arrived at by rectal and vaginal manipulation. Prognosis is usually serious. The dam and foal may perish. Treatment is similar to that in the cow.

#### Torsion of uterus in other animals

Torsion is rare in the ewe and goat. In bitch, cat and sow when torsion occurs it involves only one cornua. The affected animal shows depression, anorexia, elevated temperature, rapid pulse, tense abdomen and shows painful gait. The uterus may rupture releasing foetuses in abdominal cavity. These foetuses can be diagnosed on X-ray examination or palpation. Under such conditions hysterectomy is indicated.

#### 5. Uterine Inertia

Uterine inertia is characterised by feeble or absence of uterine contractions at or subsequent to parturition. The condition is classified as primary and secondary. Primary uterine inertia is less common.

##### PRIMARY UTERINE INERTIA

It is encountered most frequently in bitches, occasionally in cow, buffalo and sow but rarely in other species.

#### CAUSES

Hormonal imbalance particularly that of oestrogen and oxytocin at the time of parturition is the important cause of inertia.

	Oestrogen	Oxytocin
Normal Contractions	+	+
Weak Contractions	+	+
	+	+
	+	+
	+	+

The inertia might be due to primary failure of muscles to respond to hormonal stimuli or failure of hormone release necessary for uterine contractions. Individual predisposition (possibly endocrinal) was observed by Wright (1951) in eight successive parturitions in a herd of 30 Ayresshire cows.

Improper Ca : P ratio in the diet of pregnant female may cause uterine inertia since it is usually observed in hypocalcaemia. Lack of tone or degenerative changes in the uterine musculature have been observed often in cases of hypocalcaemia in Jersey cattle (Arthur, 1964).

Over-distention of uterus as observed in hydramnios, foetal giantism, foetal anasarca, twins in uniparous animals and excessive number of young in multiparous animals may lead to primary uterine inertia.

Lack of exercise, adiposity, debility and senility may lead to weak uterine contractions at parturition.

Diseased conditions of uterus and placentitis during gestation also contribute to the development of uterine inertia.

Owing to the pain in traumatic reticulitis in cows and buffaloes, the



21a Torsio uteri  
in a buffalo

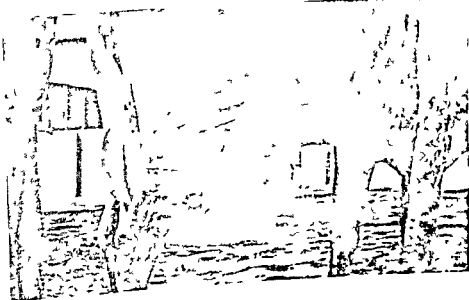


Fig A-21 Ventral her-  
nia in a cow

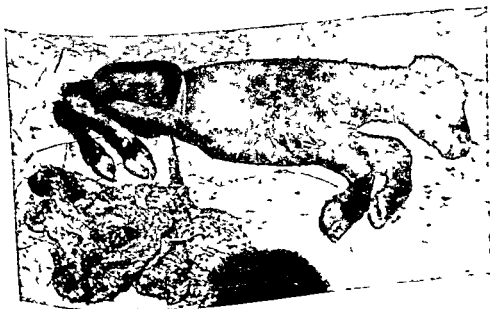


Fig 22 Death of fetus  
d.e to encircling of  
umbilical cord round  
about waist line

are not accessible for forceps delivery. In infective uteri, hysterectomy should be performed since the future breeding value of the female is doubtful.

In the case of dystokia due to uterine inertia in cats the females are usually exhausted. In such cases "feathering" is found to be beneficial in stimulating abdominal contractions. Feathering is done by pressing the finger on the dorsal aspect of the vagina and drawing it backward with slight traction. In case *this is ineffective and if the foetal presentation is normal on palpation*, oxytocin 3 to 5 u.s.p. units may be administered subcutaneously. If there is no response between 30 to 45 minutes, a second dose may be administered in conjunction with 1 to 2 ml. of 10% calcium gluconate intravenously. This is in view of the fact that calcium sensitises the uterus for the action of oxytocin by providing stronger and sustained contractions of the uterus. The use of oxytocin is contraindicated in the following conditions viz. (1) presence of abnormal uterine discharge, (2) labour exceeding 8 hours, (3) malpresentations of the foetus and (4) small or narrow pelvis. In such conditions cesarian is indicated. Forceps delivery may be attempted but is usually of little value in multiple foetuses. In emphysematous foetuses, ovario-hysterectomy is indicated.

#### 6. *Hernia of gravid uterus — Hysterocele*

The gravid uterus may slip into or be present in umbilical, inguinal, perineal, diaphragmatic or ventral hernia. It may also slip into the sac formed by the skin and cutaneous muscles in cases of rupture of the prepubic tendon. In large animals, during early pregnancy uterus does not extend sufficiently

downward and forward to result into hysterocele and in later stages of gestation it is too large to enter the hernial ring. The uterine hernia is therefore rare. Hereditary umbilical hernias are encountered in all species of animals. Gilmore (1952) attributed the cause in Holsteins to an autosomal dominant gene with low penetrance.

#### 7. *Ventral Hernia*

It is usually noticed during eighth month of gestation in mare, seventh month onwards in cow and last month in the ewe.

Predisposing causes for hysterocele include weakening of abdominal musculature which is unable to support gravid uterus apart from direct traumatic causes such as violent efforts, kicks and blows. The site of rupture is towards the ventral part of the abdomen or on either side of the linea alba, usually to the left in mare and to the right in cow and ewe. The location may also be behind the umbilicus (Fig. A-21).

In the beginning, the swelling of the size of a football occurs which rapidly enlarges to form a large ventral swelling extending from the pelvic brim to the xiphoid cartilage of sternum in front and upto the level of hocks and anus posteriorly. The whole of the uterus along with its contents passes into the subcutis. In bovines, the mass is situated between the hind limbs displacing the udder to one side. It is usually accompanied by severe oedema. Calving or foaling may become difficult on account of inability of abdominal muscles to contract. Straining is therefore not forceful to expel the foetus.

In such cases attempts should be made to deliver the young one quickly. After the necessary sedation, the animal is cast and turned on the back. The

gravid uterus slips down due to its weight and delivery may be effected in this position. Intestinal hernia which is likely to follow should be prevented.

The best recourse is however cesarian or hysterectomy in small animals.

### 8. Rupture of Pre-Pubic Tendon or Prepubic Desmorrhexis

It is most commonly encountered in mare usually in the last two months of gestation. It is rare in cows and ewes, since the sub-pubic tendon gives added support to prepubic tendon. Subpubic tendon is absent in horses (Emmerson, 1944).

The eventual lesion consists of a transverse rupture of prepubic tendon in front of pubis and rupture of abdominal muscles. It is often preceded by extensive oedema and is a serious condition.

Rupture is predisposed by increased weight on abdominal floor, degenerative changes due to oedema and weight of foetus and membranes. Violence or trauma such as severe pulling may be an occasional cause. Twins, hydrops-amnii or hydrops-allantois predispose to the rupture.

Rupture is followed by an extensive oedema extending from sternum to perineum. It is firm, painful and pits on pressure. Animal moves with caution, becomes anxious, respirations and pulse are accelerated, body is cold and she refuses to lie down. Collapse due to shock and haemorrhage may ensue.

When the eventration occurs, arch of the spine cannot be maintained leading to "lardosis or sway back". The slant of hip is increased since pubis is displaced forwards. The flanks sink and reduce the transverse diameter of body. Skin over the mass becomes tense.

Prognosis in complete rupture is grave but may become favourable if diagnosed early. Re-breeding from such females is however not advisable.

### TREATMENT

Once the rupture has occurred, the treatment is usually unsatisfactory. Prevention in the mare consists of restricting the diet and placing her in a loose box. The feed should be easily digestible. Avoid bulky feeds. A well padded bandage around the abdomen may prove useful.

If the labour pains have commenced, aid is necessary by way of traction since the force of abdominal contractions is very weak. Careful watch on the animal is essential with regard to the onset of labour pains in order to assist foetus by traction. Rolling on back or sides may help in engaging the foetus in the maternal passage. In this condition, cesarian section is of value in the cow but rarely successful in the mare.

### 9. Neoplasms

Neoplasms of vulva and vagina are rare in domestic animals. However, they may occur in all species leading to dystokia. Benesch and Wright (1951) reported papilloma, sarcoma and submucous fibroma of the vulva in cows and vaginal submucous myxofibroma in the bitches. Dystokia due to uterine leiomyoma is recorded in the Indian goat (Kaikini and Deshmukh, 1975). Cervical neoplasms are exceedingly rare and are insignificant as a cause of dystokia.

### 10. Miscellaneous causes

Other conditions which may or may not cause dystokia include remnants of Mullerian duct in the form of vertical bands in anterior part of vagina, serous

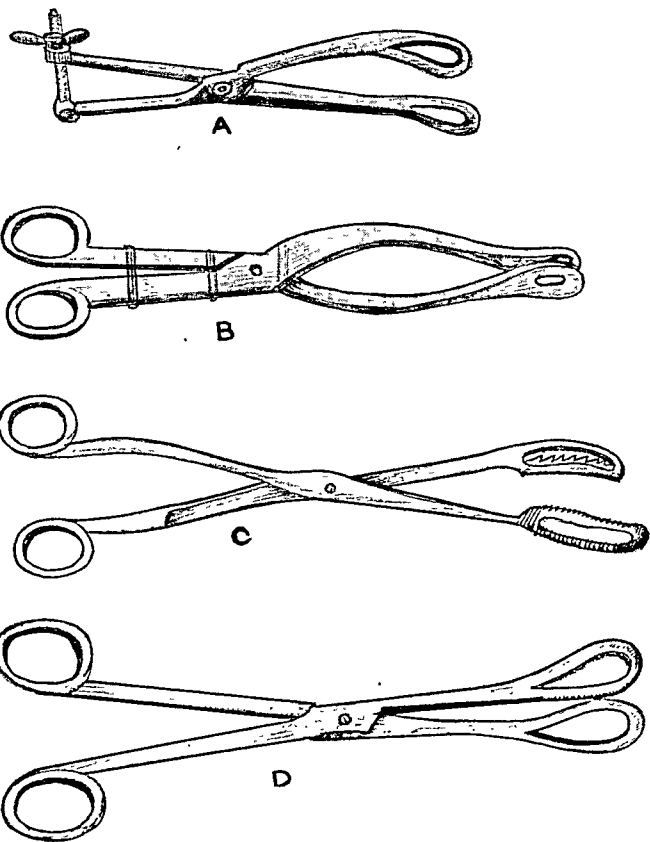


Fig. 55 Obstetrical forceps for small animals.  
A. De Bruin's; B. Moller's; C. Sewell's; D. Hobday's;

- Skjerven, O. (1965). Correction of uterine torsion in the mare by laparotomy. *Nord. Vet. Med.* 17: 377.
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cysts in vaginal wall and Nabothian cysts of cervix. The occurrence of nabothian cysts in buffaloes is reported by Sane *et al* (1969). Anatomical abnormalities like cervix bifida (Sane *et al*, 1960) and uterus bicornis bicollis (Sane *et al*, 1961) are occasionally detectable on post-mortem examination of bovine genitalia with the possibility that the animal had previous calvings. These conditions are unlikely to cause dystokia.

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reported that development of certain breeds of sheep with large heads was a common cause of dystokia. Similarly in bull-dog, Pekingese and Boston terrier, the brachycephalic and partly achondroplastic type of head is relatively a common cause of dystokia. However, Joubert and Hammond (1954) reported that this was not true in bovines. Similar findings were recorded by Arthur (1964).

Prolonged gestation has been considered to cause excess weight of foetus. Retention beyond the normal gestation period may increase the size and weight of young one from 56-80 lbs. to 117-200 lbs. in cattle. Such large size foetuses may cause dystokia.

## 2. Umbilical cord

Dystokia due to umbilical cord is very rare on account of shortness of the cord in bovines. The umbilical cord is 30-40 cm. in cow and buffalo, 47 cm. in mare, 10-12.5 cm. in dog and cat, 25 cm. in sow. The cord may be coiled around neck of the foetus resulting in asphyxia and death due to stretching of blood vessels and arrested blood supply. In bovines coiling may occur around flanks (Fig. 22).

During protracted labour it is essential to search for the cord. The tenseness of the cord is diagnostic of this dystokia. The indications for relief of such a condition include uncoiling of the cord or severing it at about 5 cm. from the umbilicus to avoid asphyxiation of the foetus.

## 3. Monstrosities

Developmental abnormalities of the ovum, embryo or foetus occur in all the species of domestic animals. The severe forms cause resorption, abortion, mummification or still-birth subsequent to

death of foetus. The less severe forms result into structural abnormalities leading to monstrosities, still-births or dystokia.

The organic deviation in either structure or form or both in one or several parts of the body is termed as a 'Monster'.

The causes of developmental anomalies are various and may be hereditary due to defective ovum or sperm or may develop due to altered uterine environment. Most of the anomalies occur in early stages of cell differentiation and the conceptus is subjected to the genetic and maternal influences. Hereditary defects are common in cattle and are due to autosomal recessive genes.

Monstrosities are common in domestic animals and more so in bovines. Of the 740 monstrosities studied by Gurlt and 71 by Saint Cyr cited by Craig (1930) the species wise occurrence is presented in Table 16 (Fig. 26. a to m).

Table 16  
INCIDENCE OF MONSTROSITIES IN  
DOMESTIC ANIMALS (CRAIG, 1930)

	Gurlt	Saint Cyr	Baumester & Rueff
Cow	239	45	0.5%
Ewe	179	16	1 in 768 lambs
Sow	87	4	—
Bitch	78	1	—
Cat	71	4	—
Mare	56	1	9 in 2340 foals
Goat	24	—	—
Mule	3	—	—
Ass	3	—	—
Total	740	71	—

These are rare in cats. These may be born with normal littermates as Siamese-twin monstrosities or without littermates also (Lunze, 1955 and Antin, 1956).

# Chapter 10

## Foetal Dystokia

Foetal dystokia is much more common in domestic animals than maternal dystokia. The pattern of foetal dystokia is more or less the same in all the species of farm animals. In foal, calf and lamb, foetal dystokia is more common due to relatively long limbs. In pig, dog and cat relatively large head is the cause of dystokia. The foetal dystokia is grouped as under.

A. Those due to physical condition of the foetus such as disproportionate size of the foetus to that of the maternal pelvis.

B. Those depending upon mal-presentation and mal-position of the foetus at the pelvic inlet.

C. Combination of A and B.

Incidence: The observations of Gudi and Deshpande (1975) are presented in Table No. 15.

Table 15  
INCIDENCE OF FOETAL DYSTOKIA IN  
BUFFALOES (GUDI & DESHPANDE, 1975)

Type of dystokia	No. of cases	% occurrence within the foetal dystokia	Overall among calvings
Abnormally large size of foetus	10	24.4%	0.6%
Deviation of head	11	26.8%	0.6%
Obstruction due to limbs	16	39.0%	1.0%
Posterior presentation	4	9.8%	0.2%

Deshmukh and Kaikini (1976) studied 86 cases of dystokia in buffaloes (Berari and Murrah) of which 64 (74.40%) were of foetal origin.

A. Foetal dystokia due to disproportionate size of the foetus. This is classified as under:

### 1. Excess in Volume of the foetus (Foetal giantism)

Dystokia due to the excess volume of the foetus either of the whole body or part thereof is commonly encountered in domestic animals.

Mating between a large sized male with a small female or cross breeding is believed to result in the large sized foetuses in few cases. In equines, Walton and Hammond (1938) reported that mating of Shire horses  $\times$  Shetland ponies or *vice-versa* did not result in increased incidence of dystokia. In uniparous animals the weight of young one is usually 6-10% of that of the dam and this rarely exceeds 1-2% in such conditions. However small bitches served by larger dogs are prone to produce remarkably large young ones resulting in dystokia. Benesch and Wright (1930) ascribed the cause of dystokia to the small litter size or reduction in the number of foetuses than to disproportion between size of sire and dam. They also

Careful examination is necessary to distinguish it from a double monster. When repulsion is applied both the foetuses move in case of monster, whereas only one moves in case of twins. The limbs may be interlocked — a condition termed as locked foetuses.

The treatment consists of dis-engaging the foetuses, application of cords to the one in favourable position and applying traction after proper lubrication. The second foetus may be corrected and dealt on similar lines.

## 5. Diseases of the foetus causing dystokla

### (i) HYDROCEPHALUS

It is dropsy of the brain and denotes slow accumulation of excessive cerebro-spinal fluid in the ventricles of brain or duramater. A large quantity of fluid accumulates in the cranial cavity causing the distension of head. The condition is common in all species (Fig. 28). Hydrocephalus is of two types — external and internal. The exact mechanism of the occurrence of hydrocephalus in man and domestic animals is not known. In the internal hydrocephalus, fluid accumulates in the ventricles of brain whereas in communicating hydrocephalus or external hydrocephalus, fluid accumulates in the sub-arachnoid space exterior to the brain. Death results due to pressure on vital centres in the brain (Smith and Jones, 1957).

Hydrocephalic tumour varies in shape and volume with a soft or hard consistency. The frontal, temporal and parietal bones are usually involved which become deformed, separated and thin like tissue paper. The skin over the mass remains intact bearing long hairs. The volume of the fluid which is colourless or yellow varies from 1 to 20 litres.

The condition does not affect the foetal development but may ensue at birth or soon after. In anterior presentation, the diagnosis of hydrocephalus is based on vaginal examination by its large size and palpation of the cephalic parts. Diagnosis is difficult in posterior presentation.

The treatment consists of judicious traction with ample lubrication, in case the mass is smaller. However in large hydrocephalic tumours it is necessary to rupture the mass manually or by trocar and canula, knife, bone forceps, chisel or Coles and Colins embryotomy knives.

### (ii) ASCITIS, ANASARCA AND HYDRO-THORAX

Ascitis is the dropsy of peritoneum. Anasarca (Water calves) is the general dropsy of tissues beneath the skin. Hydrothorax denotes accumulation of fluid in the thoracic cavity.

The causes are not definitely known but are usually ascribed to derangement of foetal circulation. It may also be hereditary or due to uterine disease. These conditions are common in bovines, but rare in other domestic animals.

**Symptoms:** On vaginal examination and uterine exploration, the ascitic foetus shows increased size of abdomen that fluctuates on pressure. The foetal size is small and it gets obstructed at pelvic inlet due to increased abdominal distention. The anasarctic foetus is characterised by roundness of the body and oedematous sensation. Head features are masked by oedema and limbs are swollen. The foetal weight may vary from 40 to 100 kg.

Prognosis depends upon the degree of effusion of fluid. Such calves are not usually born alive or may remain weak after birth.

In bovines, the common monstrosities encountered in order of frequency are:

- (i) *Schistosoma reflexus*.
- (ii) *Perosomus elumbis*.
- (iii) Double or Conjoined monsters.
- (iv) Anasarca, hydrocephalus and achondroplasia.

#### (i) SCHISTOSOMA REFLEXUS

It is found in ruminants and swine. This type of monster presents acute angulation of the vertebral column causing dorsal approximation of head and tail. The main defect is skeletal and the thoracic and abdominal tunics are absent or incomplete ventrally exposing the visceral contents (Fig. 23).

Diagnosis is based on the presence of foetal viscera, all the four legs, head and tail in the vaginal passage. The vertebral column presents a twisted appearance.

The foetus is usually born dead or rarely survives. Further breeding of the dam from the same sire is not advisable since it is hereditary.

The appropriate measures such as evisceration, traction, foetotomy or Caesarian may be undertaken to relieve the dystokia.

#### (ii) PEROSOMUS ELUMBIS

This type occurs in the ruminants and swine. Hind limbs are very rigid which is the main cause of dystokia. Primary abnormality consists of hypoplastic or aplastic spinal cord (Fig. 24). There is muscular atrophy of lumbar and sacral regions with rigidity of the joints.

#### (iii) DOUBLE MONSTERS

These consist of two foetuses joined together and are common in cow, buffalo, rare in sheep, goats and mare.

Duplicated parts may be head, body, thorax, abdomen and pelvis. Two cases of double monstrosity — *Gastrothoracodidymus octopes* in buffaloes

causing dystokia have been recorded by Velhankar *et al* (1968). The twins were united at thoracic and abdominal cavities (Fig. 25). All organs were duplicated except liver which was single. The duplication may occur either in anterior or posterior part of the body, the former being more common.

The duplicated parts and the increased number of limbs result in dystokia. Double monsters have to be differentiated from the twins and the line of treatment will vary from case to case. However manipulation, judicious traction after lubrication, foetotomy or Caesarian may be resorted to.

#### (iv) AMORPHUS GLOBOSUS — ACARDIACUS

These consist of round or spherical masses weighing 0.5 to 1 kg. and are seen attached to the foetal membranes of the normal calf. They comprise of an outer skin enclosing the mass of fat. Dystokia is rare.

#### 4. Dystokia due to twins or multiple births

In uniparous animals, the twins are usually smaller and cause dystokia when both the foetuses appear at the pelvic inlet simultaneously (Fig. 27).

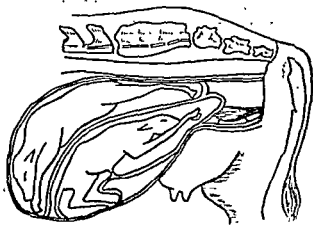


Fig. 27. Foetal dystokia due to twins. Both foetuses appear simultaneously.



Fig 23 *Schistosoma reflexus* monster in a she buffalo



Fig 24 *Nanocornus rectus* monster in a buffalo calf  
Note short straight vertebral column



Fig 25 *Gastroboracodidymus octopus* twin monster in a buffalo calf

*Indications:* If traction does not succeed, such calves are delivered by puncturing the peritoneum with suitable instruments. Amputation of one or both fore-limbs, thoracic evisceration and puncture of diaphragm may be necessary in certain cases to remove the fluid. In anasarca, deep incisions on the cutis often prove useful to reduce the foetal volume. Additional measures as suggested under excess volume of foetus may also be resorted to.

### (iii) CONGENITAL OCCLUSION OF URACHUS

A rare condition of excessive accumulation of urine in the bladder as a result of congenital occlusion of urachus has been described by Craig (1930). It causes enlargement of abdomen and dealt with as in ascitis.

### (iv) POLYSARCIA OR LARD CALVES

Polysarcia is the accumulation of excessive quantities of fat in the subcutaneous tissues. These have been described by German obstetricians only. The foetus may weigh upto 50 kg. Relief measures are similar to those for excess volume of foetus.

### (v) TUMOURS

Tumours of the foetus are a very rare cause of dystokia. These may be of varying types situated on or within the body of foetus and include teratoma, hygroma, fibroma, papilloma and cysts. It is often necessary to excise the tumour, if well defined rupturing the cyst to evacuate its contents can be tried.

### (vi) CONTRACTIONS

Permanent contractions of the muscles, tendons, ligaments causing abnormal direction of foetal limbs and neck may lead to dystokia. These have been described by Craig (1930). Usually the neck and limbs are affected.

The causes are obscure and this condition may be due to faulty intra-uterine position of the foetus over a long period, pressure due to viscera — particularly colon as in the mare and diseases of nerve centres. This condition may occur during early gestation.

In foals, the neck is usually involved on account of long neck and the condition is called as 'wry neck'. The neck is rigidly curved to one side and any attempt to straighten it is futile. When the limbs are involved it gives a distorted shape and these cannot be straightened.

Extension of limbs or neck by tenotomy may be tried to straighten the affected part. However, this is very doubtful and foetotomy is the only alternative.

### (vii) EMPHYSEMA

It is the type of putrefaction characterised by formation of gases in the subcutis within 24 to 72 hours subsequent to death of the foetus. The foetus becomes soft, decomposed and distended with gases.

Emphysema ensues in all conditions causing death of foetus or uterine inertia. It is also observed in prolonged cases of uterine torsion and septic metritis. In unicornual twins after expulsion of one foetus, the other may be retained leading to its death and emphysema. In larger breeds of dogs, the last pup may remain undetected, die and becomes emphysematous.

*Symptoms:* Suspicion of emphysema should always arise in protracted cases of dystokia extending over 24 hours. The dam appears depressed and an-orexic with elevated or subnormal temperature. Heart rate and respirations are usually accelerated and labour is weak and intermittent.



Fig 26 g *Dipygus bidorsualis*



Fig 26 h *Ectopia cordis*

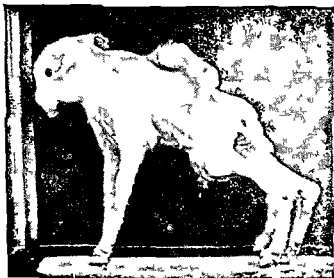


Fig 26 i *Opisthomelophorus trichurus* with anterior limb on neck

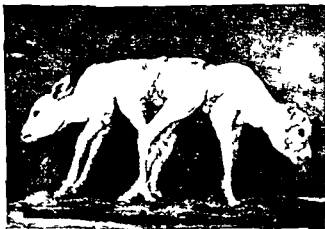


Fig 26 j *Octopus janus* Two faces placed opposite each other with eight limbs



Fig 26 k Hernia of brain (encephalocoele)

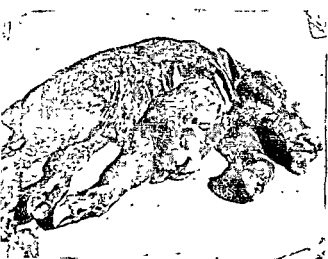


Fig. 26 a. *Acephalus acardiacus*

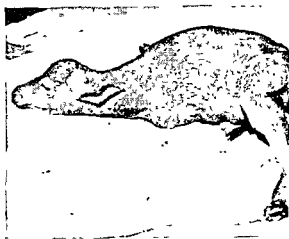


Fig. 26 b. *Peromelus achirus*.



Fig. 26 c. *Monocephalus dibrachius*. Note two fore limbs and six hind limbs.



Fig. 26 d. *Dicephalus subbicollis*.



Fig. 26 e. *Dicephalus omocephalus*. *bicollis*



Fig. 26 f. *Epigastrodidymus octopes* (*Dicephallus*)



On vaginal examination, foetid watery discharge may be noticed. The vaginal mucous membrane is usually dry, swollen and inflamed. On uterine exploration, the walls appear to contract tightly around the foetus and the cervix is involuted or contracted.

The foetus is dead and the extremities are cold to touch. Crepitant feel of the subcutis is palpable. The foetal skin is dry, covered with meconium and the foetal fluids are scanty. Hairs and teeth become loose.

**Prognosis:** Prognosis is guarded due to possible complications such as metritis and metro-peritonitis.

**Indications:** If the foetus is in proper presentation and position, gentle traction may be applied after adequate lubrication of the maternal passage. Traction is little easier in cases of emphysema since even pressure is exerted on all parts of the birth canal by subcutaneous gases. Failing this, forced traction after correction may be necessary. This is aided by deep incisions on the skin to relieve the gases.

Reduction of volume is easily accomplished by foetotomy which is much easier in emphysematous calves due to soft and friable tissues. Caesarian is indicated as a last resort. It is advisable to perform hysterectomy in bitches. After relieving emphysematous foetus it is essential to treat the uterus with antibiotics.

#### B. Foetal dystokia due to malpresentation and malposition.

Various types of foetal dystokia due to abnormal presentation, position and posture are encountered in all the species of domestic animals. These are probably due to reduced viability of the young one. Failure of rotation of the foetus from intrauterine position to the

normal parturient position may result in dystokia. On its way through the birth canal, the part of foetus — limb or chin — may get stuck up or engaged at the pelvic brim leading to further continuous flexion of the part and subsequent dystokia. Dystokia is common in all the transverse presentations and in adhesions of foetus and foetal membranes when the normal presentation cannot be attained.

Usually the normal presentation and position assumed by the normal foetus at the time of delivery is the anterior presentation, dorsosacral position with extended forelimbs and head resting on metacarpal bones. Presentation or position other than this, is likely to cause dystokia. The abnormal presentations and positions are shown in Table 17.

#### Dystokia due to postural defects

Postural defects in anterior presentation:

In order of frequency the postural defects in ruminants occur next to foetal oversize. Of these, the carpal flexion and lateral deviation of head are the commonest forms. These defects can readily be corrected by manipulation if treatment is resorted to in the beginning of second stages of parturition. These will be considered now in details.

##### 1. ANTERIOR PRESENTATION

##### (a) *Dystokia due to abnormal positions:* (Fig. 29, 30).

All positions except normal anterior longitudinal presentation require assistance at birth (Table 19). The main steps involved in correction are retropulsion correction—forceps or manual grip and manual or mechanical rotation of the foetus in order to bring it to dorsosacral position — and exerting traction



Fig. 26 l. Foetal monster with short upper jaw and protruded tongue.



Fig. 26 m. Cyclops monster.



Fig. 28. Hydrocephalus in a buffalo calf

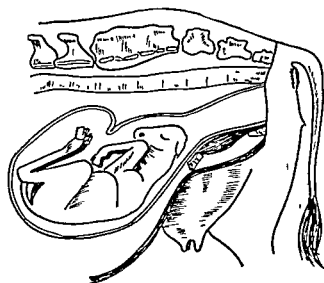


Fig 29 Foetal dystokia due to dorso pubic position

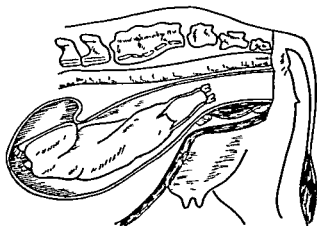


Fig 30 Foetal dystokia due to right dorso iliac position.

thereafter. Failing which foetotomy or cesarian is indicated

(b) *Dystokia due to hind limbs in anterior presentation:*

- (i) Hind limbs are extended and retained at stifle (Hip lock):

Interlocking of maternal and foetal pelvis occurs in anterior presentation. The head and thorax are out of pelvic brim but the stifles are obstructed.

To rectify the position it is necessary to push back the foetus to disengage the pelvis and apply oblique traction so that one stifle clears the pelvic brim first. Failing this, detrunca- tion, abdominal exsiccation and sometimes bisection of the pelvis may be resorted to.

- (ii) Flexed hind limbs pass into the genital canal ("Dog sitting position")

It is a very rare condition met with in the bovine. It is very formidable and dangerous type of dystokia. The diagnosis is based upon the presence of head, both forelimbs and hind limbs under the body in the birth canal. Back of foetus is jammed against maternal sacrum. Hind limbs may penetrate

uterus leading to its rupture and severe haemorrhage.

The indications for relief include re- tropulsion with adequate lubrication of hind limbs beneath the body far beyond the pelvic brim. This is usually not successful. Conversion of the presentation to anterior or posterior one may be required. If the foetus is dead, detrunca- tion or division of body at thorax and abdomen may be of use. In serious cases, cesarian is indicated.

(c) *Abnormal anterior presentation, dystokia due to deviation of fore limbs:*

This is a relatively common cause of dystokia in unipara on account of long and rigid limbs. When a portion of forelimb is caught in the pelvic inlet, the leg is forced backwards towards the body flexing the shoulder and elbow joints resulting in dystokia due to in- crease in the pectoral diameter. In multipara, the limbs are short, flexible and remain in close apposition to the body and therefore do not cause dystokia. Four principle types of dys- tokia due to fore limbs include:—

Table 17

## DYSTOKIA DUE TO MALPRESENTATION OR MALPOSITION OF THE FOETUS

	Natural	Causes which may be met with in all positions	Abnormal position	Dorso-pubic Dorso ilial (Right or left)
Anterior presentation	Natural	Causes which may be met with in all positions	Obstacles due to hind limbs	<ul style="list-style-type: none"> <li>Hindlimbs extended and retained at the stifles.</li> <li>Hind limbs flexed beneath the body and simultaneously entering the pelvis</li> </ul>
			Obstacles due to fore limbs	<ul style="list-style-type: none"> <li>Limbs on the neck</li> <li>Limbs partially extended in the pelvis.</li> <li>Limbs flexed at knees</li> <li>Limbs completely retained.</li> </ul>
			Obstacles due to the head	<ul style="list-style-type: none"> <li>Head flexed beneath the chest.</li> <li>Head turned to one side of the body.</li> <li>Head extended on the back.</li> <li>Head flexed downwards.</li> </ul>
			Combined obstacles due to the head and limbs	<ul style="list-style-type: none"> <li>Head retained with one or both fore limbs.</li> <li>Head retained with one of the fore or hind limbs in the pelvis.</li> <li>Dorso pubic.</li> </ul>
Posterior presentation	Natural	Causes to be met with in all positions	Abnormal position	<ul style="list-style-type: none"> <li>Dorso-ilial (right or left).</li> <li>Neck contracted.</li> </ul>
			Obstacles due to the head or fore limbs	<ul style="list-style-type: none"> <li>Fore limbs under the chest entering the pelvis.</li> </ul>
Transverse presentation	Natural	Causes to be met with in all positions	Obstacles due to the hind limbs	<ul style="list-style-type: none"> <li>Limbs partially extended in genital canal.</li> <li>Limbs flexed at the hocks.</li> <li>Limbs completely retained.</li> </ul>
			Dorsal position Ventral position	<ul style="list-style-type: none"> <li>Cephalo ilial—right or left.</li> <li>Cephalo sacral — vertical</li> <li>Cephalo ilial — right or left</li> <li>Cephalo sacral — vertical.</li> </ul>

limbs brings about this condition. One or both limbs may be involved. The metacarpus is flexed on the radius, radius on humerus and humerus on scapula so that the whole limb appears folded. Shoulders are pushed backwards and lie against the thorax. This increases the transverse and vertical diameter of thorax resulting in dystokia (Fig. 33).

The palpation of flexed knee joint on vaginal examination is a diagnostic feature.

It is necessary to raise the hind parts of the dam to facilitate correction of the posture. Suitable and ample lubrication is indicated. Introduction of warm water mobilises the calf to a certain extent. Epidural anaesthesia prevents straining and is useful for easy correction. The head may be secured by sterilized nylon cords and the foetus is repelled to create space for correction. Depending on the limb involved the corresponding hand may be used. If the left limb is to be corrected, the foetus is repelled in right flank and vice-versa. The knee joint is raised high as much as possible. The hand is passed below and the metacarpal bone is seized and raised still higher. The hoof is now grasped in cup-shaped hand and strongly flexed on the fetlock and this flexed portion is guided

out of pelvis. Once the limb is straightened it is corded securely. The other limb is also treated similarly and delivery effected with or without traction.

If the foetus is small, cords may be fixed around knee joints and head and forced traction done to effect delivery. However, as far as possible this method should be avoided.

Failure to correct this condition, foetotomy consisting of decapitation and amputation of both fore limbs at knee joint or shoulders is indicated. If this posture is accompanied with abnormal position, the foetus has to be rotated to dorso-sacral position before applying traction.

(iv) One or both forelimbs completely retained; shoulder flexion or shoulder presentation:

The shoulder flexion is common in the mare than in the cow. According to Craig (1930) death of foetus is the rule in shoulder presentation.

The obstacle to birth is due to shoulders being fixed against the thorax which increases transverse diameter and also the vertical. Radius gets fixed in the cotyloid fossa of humerus and posterior flexion of elbow is not possible. One or both limbs may be involved (Fig. 34). On vaginal examination only head is

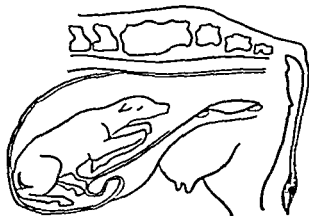


Fig. 33. Foetal dystokia due to carpal flexion.

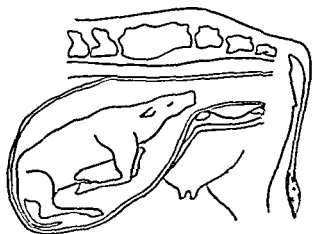


Fig. 34. Foetal dystokia due to shoulder flexion.

- (i) One or both forelimbs crossed on neck: (Fig. 31).

This condition is common in mare, less so in the cow. The shoulders are not lodged in the hollow space on the sides of neck and are fixed on the chest, thus increasing transverse diameter. The diagnosis is easy on vaginal examination, feet being displaced upwards. The straining is usually violent and the dorsally directed hooves may rupture the uterus, vagina or rectum.

It is necessary to secure the limbs above the fetlock joint in bovines and pastern joint in equines, raising it upwards then laterally downwards and medially till it is below head and neck. Head may be raised a little higher. When both limbs are found in crossed posture over the neck, the foetus may be repelled after securing the limbs. For correcting left limb of the foetus it is convenient for the obstetrician to use the right hand and *vice-a-versa*. Application of proper traction is necessary to complete delivery.

- (ii) Incomplete extension of fore limbs, flexion of anterior limbs at elbows, or incomplete extension of elbows: (Fig. 32).

This condition is very common in cows. The forelimbs do not progress

forward along with head, the muzzle appears to be resting on fetlock and hoof, instead of on the metacarpal bones. The elbow joints lie along side of thorax thus increasing the pectoral diameter. Olecronon processes of ulna are impinged at the pelvic brim.

Forced traction after cording the head and limbs may be tried. In other severe forms it is necessary to secure the head and forelimbs and repel the foetus forward in the uterus. The obstetrician cups the olecronon the force being applied simultaneously. The object is to straighten the limbs at elbow.

- (iii) Forelimbs flexed at knee, knee flexed posture or carpal flexion:

Of the dystokias due to pathological postures, this type is the commonest in cow, buffalo and mare. The condition is not so important in ewe, goat, bitch, cat and sow since the metacarpal bones are small and do not offer any obstacle to birth.

Carpal flexion results when hoof of the foetus gets impinged against the pelvic brim. With the progressive straining and as the foetus advances, the limb gets gradually flexed at knee joint. The foetus in intra-uterine position lies with forelimbs flexed. Death of foetus with failure of rotation and extension of

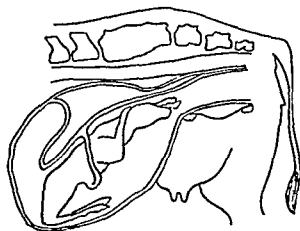


Fig. 31. Right fore limb of foetus crossed over the neck.

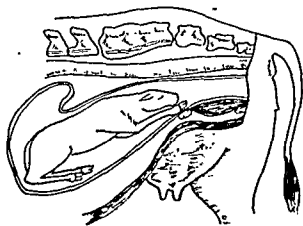


Fig. 32. Incomplete extension of fore limbs.

inlet can be identified in the mare by the mane above and trachea below. The head is easily recognised by ears, eyes and nostrils. Usually the foetus is in the dorso-sacral position.

*Indications:* The aim in correction of this deviation consists of adjusting the head and bringing it in favourable position. The hind quarters of the female are raised. Standing position is always preferable but in recumbency, the animal should be placed on the opposite side of deviation and lubricated hand is introduced in the vagina. On the cessation of provoked straining, the foetus is repelled by application of pressure at the base of the neck, preferably on the opposite side of the deviation. One hand is transferred to the muzzle, nostril or jaw and it should be brought round through an arc until the nose is in line with the birth canal. In such conditions, use of Harris or Krey Schottler's hook is advocated. While attempting repulsion, care has to be taken regarding the expulsive efforts. In such cases epidural anaesthesia is indicated and the drained foetal fluid should be substituted so as to increase the intra-uterine mobility of the foetus. A special head cord of smaller calibre in the form of a running nose is passed on to the mandible and the snare pulled at by the assistant with simultaneous repulsion to assist correction.

Forced traction may be tried in some cases. Care should be taken to empty the rectum. Serious danger of uterine and perineal rupture may be anticipated. In still more obstinate and delayed cases or the so called wryneck condition, correction becomes impossible and decapitation may be resorted to by means of foetotome. The decapitated head is re-

moved by Krey Schottler's hook and the rest of the body may be withdrawn by traction on forelimbs. Occasionally removal of one limb on the opposite side of deviation creates space for manipulations.

In mares, this is a serious malposture and the deviated head is often beyond the reach. Narcosis by Chloral hydrate and epidural anaesthesia is indicated. A snare is introduced by Schriever's introducer around the neck and fixed to the Kuhn's crutch. The crutch is advanced further by to and fro movements and the snare tightened. Pressure is applied on the pectoral muscles and the snare is pulled out simultaneously. The foal's head then comes into easy reach of the operator. The muffle is guided and brought into the genital canal. Blanchard's long handled hook can be applied to the foal's orbit, mouth commissure, nostril or ear canal. Decapitation by the use of Persson's saw and Thygeson's foetotome is only solution in Wryneck condition.

In ewes and goats the deviation can be corrected by short and blunt finger hook and applying proper traction. In bitch and sows, the hind limbs of the dam are raised, lubricants introduced and the foetus is pushed back with fingers. Traction by hooks or forceps accompanied by abdominal manipulation may be useful. Caesarian may be performed as a last resort.

(ii) *Downward deviation of the Head* (Butt or Poll or Vertex presentation) — Type one —

These are of two types. The nose of the foetus is towards the trachea and the poll is presented at the inlet. The foetus is usually in the dorsosacral position, whereas in type two cases, the entire head is dropped down between the

visible. With every act of straining the head makes little progress and recedes back since the shoulders are held at pelvis. Severe straining ensues but there is no progress at birth.

The object of correction of this abnormal posture consists in conversion to carpal flexion. Repel the foetus forwards and upwards. After securing the head pass a running nose or cord around the forearm and apply traction. It will be possible to bring the limb into a carpal flexion. Once this is achieved it can be treated as indicated above.

When only one limb is retained and retropulsion is not possible, forced traction after suitable lubrication may be judiciously tried. In case this is not possible, decapitation may be done in order to get more space. The foetus is repelled, limbs are corrected and delivery effected. In some cases it may be necessary to amputate one or both forelimbs at shoulder by a foetotome.

Cesarian may be done as a last resort.

#### (d) *Obstacles due to head:*

This is more frequent and more serious cause of dystokia in ruminants, than dystokia due to limbs. The tendency of the head becoming displaced varies with the species. The long and flexible neck of the foal is very prone for such deviations. It is less so in calves and least in pigs and dogs due to short and thick neck. The deviation may occur prior to or long before parturition. Head deviation may or may not be complicated by those of limbs.

#### (i) *Lateral deviation of head:*

This is the commonest type of dystokia in ruminants. The incidence is much more in primipara. Saake (cited by Craig 1930) reported 80 out of 108 (74.07%) pregnant animals with this type of dystokia. The lateral deviation

may occur, either to the left or right, constituting the left lateral and right lateral deviation.

During parturition, if the os is closed or not properly relaxed and in the absence of active role of the foetus, it cannot be forced through the genital canal with every contraction. The nose or some other part of the head may become deflected or when the os is closed the muzzle glides on to the os to one of the sides. With the progressive contractions of the uterus, the deviation further increases leading to left or right deviation (Fig. 35). Ultimately the neck becomes doubled directed backwards and the head rests along the chest in bovine, and along the flanks in the equine. Left lateral deviation of the head is more commonly encountered in cows and buffaloes.

When the deviation is slight, delivery can be accomplished without much assistance. The prognosis is serious when the foetus is dead and the deviations are due to muscle contractures. Diagnosis is easy and on vaginal examination, both the forelimbs are felt in the passage. In the right lateral deviation, right limb is less advanced and *vice versa* but no portion of the head is palpable. The convex mass facing the

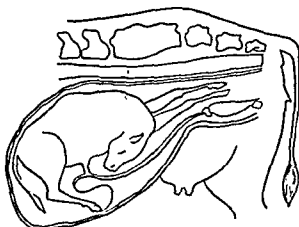


Fig. 35. Right lateral deviation of foetal head.



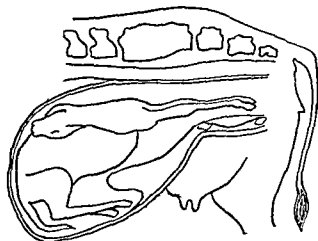


Fig. 37. Dystokia due to upward deviation of foetal head.

Cesarian may be performed in bitches in good time.

## 2. DYSTOKIA IN POSTERIOR PRESENTATION

Due to long and rigid limbs in unipara birth in posterior presentation is usually serious. The dorso-sacral position with both the hind limbs completely extended is possibly the only combatible position wherein delivery without assistance may be possible. Such cases are however very few and posterior presentation usually results in dystokia and higher incidence of foetal mortality is due to asphyxiation subsequent to the rupture or compression of umbilical cord. About 4 to 5% dystokia are due to the posterior presentation.

### (a) Dystokia from abnormal positions:

(i) Dorso pubic position: In this position the foetus lies on its back and the feet and belly are directed towards the maternal sacrum. The thighs and buttocks form a voluminous mass leading to dystokia and there is a danger of the foetal limbs lacerating and rupturing the vagina or rectum. In order to correct this position, it is essential to rotate the foetus converting the position

into dorso-sacral one. Rotation is much easier in posterior presentation since the croup and rump is round instead of conical withers as in anterior presentation. It is advantageous to the obstetrician, since the obstetrician has not to attend to the head and forelimbs. Both hind limbs may be corded and pulled separately backward and downwards in order to bring the os calcis in the canal. Later the traction is directed upwards to raise the croup, followed by steady traction during labour. All the manipulations have to be done very carefully to avoid accidental perforation of the uterus. Epidural anaesthesia, proper lubrication, traction with application of anal hook, retropulsion greatly assist the correction. In delayed cases, foetotomy or caesarian are indicated.

### (ii) Dystokia due to dorso-iliac position

These pathological positions may be handled as in the above case, the ultimate aim being to convert these positions into dorso-sacral one.

### (b) Dystokia due to head or forelimbs:

Dystokia due to head (as in muscle contracture) or one or two forelimbs along with the posterior limbs are very rare causes of dystokia. The manipulative correction consists of repulsion of the part or its excision.

### (c) Deviation of hind limbs:

#### (i) Hind limbs incompletely extended in the genital canal:

This is a rare cause of dystokia and usually results due to the impinging of the stifles at the pelvic inlet. This accident is much more common in mare and less so in cow, probably due to sloping border of the pubis. Once the hind limbs are flexed the foetal body continues to pass outwards with the result that femurs become more vertical, the croup is raised against the lumbar

forelimbs. (Nape or breast head posture presentation — Fig. 36).

When the head of the foetus is not in the axis of the pelvis but inclined downwards, the nose may become impinged against the brim of the pelvis. The condition aggravates with further uterine contractions and the neck becomes more and more flexed so as to lie horizontally on floor of the pelvis and the lower jaw lies against the trachea. Diagnosis is easy on manual exploration and in the first type, correction is much easier. The forehead of the calf, is repelled by the thumb and the mandible is lifted over the pelvic brim by the fingers. Alternately the obstetrician passes the hand along the floor of the pelvis and the nose is received in the hollow of the palm. The wrist is flexed and the nose drawn towards the vulva. Introducing the hand in the mouth or fingers in the nostrils and a running cord around the upper jaw, may prove useful.

In the second type, considerable difficulty is experienced and it is necessary to repel one or both forelimbs inside the uterus. This creates space for manipulation of the head. The leg is re-extended and delivery effected by traction. Alternately, foetus may be rotated by means of Cammerer's torsion fork

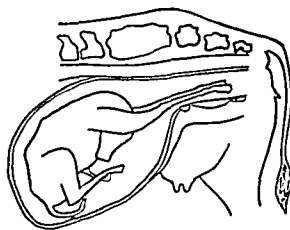


Fig. 36. Dystokia due to downward deviation of foetal head.

converting the position into a ventral one, when the head may be easily extended. Foetotomy may be resorted to when manipulation is not possible. It consists of removal of the head or one of the forelimbs. In the obstinate cases of deviation and when the calf is alive caesarian is indicated.

Downward deviation is quite common in mares than in cows. Nape posture is the commonest. The use of mandibular snare proves to be a great asset. Spontaneous delivery of the foal in nape posture is possible and therefore when the ears are visible at the vulva forced traction without correction may be tried.

In ewes and goats, the use of hooks and forceps may be necessary for corrections.

### (iii) Upward deviation of the head:

Upward deviation of head is rare in the uniparous animals, but since in the carnivora the broad head and neck are flexible in the dorsal direction, it is common in bitches and cats. It is occasionally observed in mares and may lead to rupture of uterus and rectum and foetus expelled through recto-vaginal fistula. The foetus is usually in dorso-sacral position. The forelimbs are in the vagina, sternum of the foetus is presented and the trachea is felt upwards. The lower jaw faces the sacrum of the dam and the head may be inclined on withers or on either side (Fig. 37). The diagnosis in uniparous animals can be done on manual exploration and in multipara by digital exploration. In order to correct this deviation, the repulsion is continued on the sternum while the lower jaw is seized by hand or cords and the head is brought in the genital canal. The use of hooks in the orbit may be tried. Foetotomy is rarely necessary and consists of either decapitation or decollation.

It is a posterior longitudinal presentation, dorso-sacral position, bilateral hip flexion posture. This is frequently observed as a cause of dystokia in domestic animals — more so in the mare. Both the hind limbs are extended forwards upon the pelvis beneath the abdomen and chest of foetus, with each joint rigidly extended. The causes of this condition are similar to those for the hock flexion posture. In fact many cases of hock flexion terminate in this condition. The flexed hocks may be impinged on the pelvic brim as the foetus is pushed towards the vulva. Os calcis glides downward and forward and the hock joint becomes extended and finally comes to lie beneath the body (Fig. 39).

#### Diagnosis:

Diagnosis is arrived at by the presence of tail and buttocks being in the pelvic cavity. At times haunches may be felt but the hocks are beyond reach. The degree of engagement of the foetus in the maternal pelvis varies. It is difficult to recognise any part of the foetus unless repulsion is done.

Indications for relief of this condition primarily include the conversion into a hock flexion posture. After the preliminary arrangements such as raising of hind quarters, epidural anaesthesia and

proper lubrication, the foetus is repelled at the perineum with hand or Kuhn's crutch in a forward and upward direction so as to bring the hock nearer to the operator. When the traction is applied on the hock by a snare or hand, it is converted into a hock flexion posture. It should then be dealt with as described earlier. Forced traction without adjustment is usually serious but may be tried in case the foetus is small and pelvis capacious. This can be accomplished by passing a cord around the thigh or around the abdomen in front of thigh and applying traction on cord and tail. Alternatively, hook may be fixed in the muscles of the croup. This is risky since the hooks are likely to slip. Another method consists of incising the perineal region of the foetus and fixing the Obermeyer's anal hook at the symphysis, ilium or obturator foramen. Forced traction can be effected. Foetotomy proves highly effective particularly in mares, when the foetus is dead and emphysematous and when manipulative corrections are not possible. Removal of one or both hind limbs at the hip joints should be the aim, the wire being passed around the stifle joint. It is necessary to include the tail into the loop of wire and the head of foetotome rests on the perineum during embryotomy. By this method the femur can be sectioned at the acetabulum. At times, removal of only one limb alone facilitates correct manipulation of the other limb. Epidural anaesthesia and raising the hind quarters of the mare or cow greatly assist the operation. In ewe and goats, where the hock and the hip flexion postures are common causes of dystokia particularly when twins are present and may be corrected by manipulations as described earlier. Cases in which correction is not easily accomplished, amputation at the hock or hip

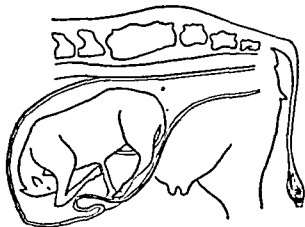


Fig. 39. Foetal dystokia due to Breech presentation.

region of the mother and finally is jammed.

#### Diagnosis:

Diagnosis of this condition is arrived at from the soles of hind feet which are uppermost. The hocks are flexed, stifles butt against the pubis and the croup is pressed on the sub-lumbar region. Invariably the tail is felt in the vagina.

#### Correction:

It is necessary to apply cords to the hind limbs and repel the croup in-between the labour pains. Simultaneously, traction is applied on the cords in order to extend the limbs completely.

- (ii) Hind limbs flexed at hocks or flexion of posterior limbs at the tarsus or hock flexion posture:

This is a comparatively common condition. The incompletely extended hind-limbs of the foetus come in contact with pubis and the foetus is propelled forwards; the canon bones get pressed against the thighs. The ultimate result is the folding of the whole limb, the phalanges being flexed on metatarsus, metatarsus on tibia, tibia on femur and femur on pelvis. In addition to this the hooves are engaged on the pelvic brim and act as hooks (Fig. 38).

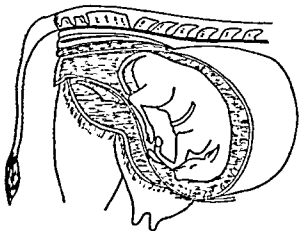


Fig. 38. Dystokia due to hock flexion.

#### Diagnosis:

Diagnosis of this condition is easy and is based upon the palpation of the hocks, tail and ischial tuberosities. In sheep, bitches, and sows, the foetus may be extracted forcibly after adequate lubrication. However, in the cow and mare, forcible extraction may cause laceration and rupture of the genitalia.

The aim of the manipulative procedure is to extend the hock or hocks. The condition is usually bilateral. After epidural anaesthesia the foetus is repelled forwards so as to create space in the pelvic cavity. The hoof is grasped in the palm and flexed strongly on the pastern and fetlock. It is pulled medially and the foot is drawn back through an arc. At the same time the hock is being firmly flexed and retropulsion maintained. The hoof is lifted over the pelvic brim and extended in the vaginal passage. Manual extension may be assisted by traction on a cord applied to the foot. In case the hocks are not easily accessible a running noose may be passed with the help of Schriever's introducer and when pulled, the hock comes in easy reach. This is followed by correction of the foot. The manipulation can be done easily in standing position and it is convenient to use left hand for the left limb and *vice-versa*.

Forced traction may be tried by applying the anal hook in the birth canal. Easier flexion of the limb may be achieved by severing the Achilles tendon. Foetotomy occasionally becomes necessary which is simple and consists of amputation of hind limbs at the hock joint. Caesarian is the method of choice in small animals.

- (iii) Thigh and croup presentation (Breech presentation). Complete retention of hind limbs or hip flexion posture:

such a case is to achieve version of the foetus which is effected by administration of epidural anaesthesia, raising the hind quarters of the female, introduction of emollients and taking grip of one of the extremities by hand, cords or Krey's hook and repelling the opposite extremity. Thus the transverse presentation is converted into a longitudinal one and after the proper adjustment of position and posture, foetus may be delivered by gentle and steady traction. Should manipulative correction fail, foetotomy may be resorted to by dividing the lumbar region, thoracic and abdominal evisceration and removal of each part.

In smaller animals, version may be possible by forceps or external manipulation. Caesarian is indicated in refractory cases.

#### (b) DYSTOKIA DUE TO VENTRAL PRESENTATION

This type of presentation is more common particularly in the mare. The head and varying number of limbs are present in the pelvic inlet. The oblique variants are observed and the foetus may be horizontal (right and left cephalo-iliac) or vertical (cephalo-sacral). The condition can be diagnosed from sternum, abdomen, head and limbs present in the pelvic inlet (Fig. 41). This should be differentiated from the double monsters, twins and *Schistosoma reflexus*. The condition is not very troublesome since the foetal parts can be easily reached. Manipulative corrections consist of converting the transverse presentation into longitudinal, preferably a posterior one. Hind limbs may be subjected to traction after application of cords and repelling the cranial extremity by Kuhn's crutch. Epidural anaesthesia and copious amounts of emollients may

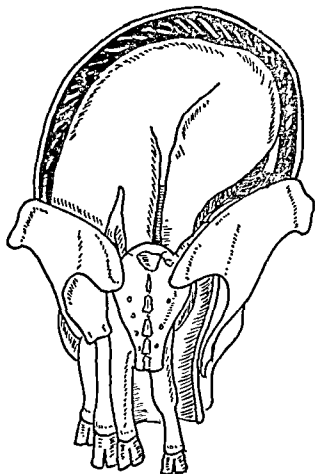


Fig. 41. Foetal dystokia due to transverse ventral presentation.

be necessary for cow and deep narcosis for the mare. After converting the position into longitudinal presentation, dorso-sacral position, foetus may be extracted by gentle traction. Foetotomy is likely to be laborious and consists of amputation of limbs and traction after correction. Lowering the hind parts of the dam using slopy floor or keeping her on back may be useful. Should the foetal version be impracticable, caesarian is recommended.

#### 4. The compound or rotated bicornual pregnancy

Pauli (1842) was the first to record compound or rotated bicornual pregnancy. Williams (1889) recorded three cases in pluriparous mares. Fincher

joint may be carried out by wire saws used in conjunction with Glatili's spiral tubes. Forceps, smaller hooks or tube noose may be used in smaller animals to effect delivery by forced traction. Excessive traction in multipara should be avoided. It is advisable to perform caesarian in small animals.

### 3. Dystokia due to transverse presentation

Transverse presentation even though rare is reported from all species except in swine. In transverse presentations, the long axis of the foetus is at right angles to the maternal axis. Transverse presentation can be of two types:— (1) Transverse dorsal and (2) Transverse ventral, depending upon the dorsal or ventral part of the foetus presented at the pelvic inlet.

These presentations are not usually primary and arise as a result of strong uterine contractions particularly when the cervix is closed. These are associated with conditions such as torsio uteri, spasms of the cervix, deviation of head, deformed head and hydrocephalus. Dystokia arising from such presentations is serious and the aim in overcoming them is to perform version of the foetus so as to convert a dorsal or ventral presentation into a longitudinal one. It is preferable to convert it into posterior longitudinal rather than anterior longitudinal presentation since only two foetal parts are required to be manipulated in the former and three in the latter.

#### (a) DYSTOKIA FROM TRANSVERSE DORSAL PRESENTATION

This is very rare in cattle when the dorsum of the foetus becomes convex and faces the cervix. John (cited by Williams, 1913) reported that transverse pregnancy occurs in about 4% of

dystokia cases in mare. Rare cases of bicornual pregnancy result into transverse presentation. In the ewe it may occur when triplets or quadruplets cause great distension of the cornua. Due to the peculiar position of the uterine cornua which meet at a very acute angle, transverse pregnancy is very rare. This condition is occasionally seen in carnivora while one foetus is engaged in birth canal and the second in turn may be forced across the uterine body in the opposite cornua. Transverse pregnancy in carnivora is rare and may be encountered with single foetus in bicornual pregnancy.

Dystokia due to transverse presentation can be diagnosed from the foetal back presented at the inlet, withers, mane in case of the foal, lumbar region and ribs. The labour pains are continuous, water bags have ruptured, straining is good, but no foetal part appears at the vulval opening. The head and limbs are usually out of reach. Three variants of this position are possible and the foetus may be horizontal (right and left cephalo-iliac) or vertical (Cephalo-sacral) (Fig. 40).

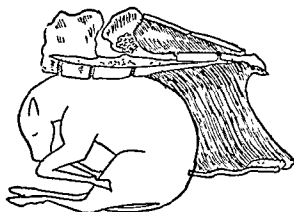


Fig. 40. Foetal dystokia due to transverse dorsal presentation.

Even though the pelvic inlet is free of the foetal parts, this type of dystokia is of a serious type. The aim in relieving

# Chapter 11

## Lethal Factors

In Livestock improvement, the first line of defence must be based on the inherent fitness of the animal to live, function and resist the adverse conditions it may be subjected to, by the demands and the environmental limitations of domestication. Lethals are genetic factors that cause death of the individual in which they occur. Death takes place either during prenatal life or shortly after birth. The lethal factors can alter the conformation and function of the neonate so that it does not survive. Most lethals appear at birth in view of the greatest adjustments the animal has to make from pre-natal to post-natal surroundings. Severe adjustments include use made by the heart, lungs, digestive mechanism and thermo-regulations.

The term 'Sublethal' is frequently used to include conditions that represent less drastic physiological or anatomical incompatibilities than the lethals. As a result, animals with sublethal factors may be deformed but do not die. Some of the lethals affect the foetus after pregnancy has been fully established while others interfere with early phase of embryonic development. Some of the early foetal deaths are associated with such genetic factors. In early foetal death, the conceptus may be aborted or become mummified but in the event of foetal death just before birth it may

interfere with normal parturition. Foetal malformations may also occur as a result of the transmission of toxic substances, viruses, hormones and antibodies from the dam to the foetus and also through deficiencies and surplus of specific nutritional factors in the maternal diet during pregnancy. These malformations which are not inherited, may also resemble those genetic in origin and are called 'Phenocopies'. Phenocopies also occur in cattle in certain cases of cleft palate, congenital dropsy, muscle contractures and ankylosis (Bane, 1964).

Lethal factors have long been recognised in farm animals and were described as monstrosities or "freaks of Nature". Much of the information regarding lethals has come from Scandinavian countries. Lerner (1944) classified lethals by grouping them anatomically according to the tissues chiefly affected, viz. bone, connective tissue, muscle, epithelium, nerves and blood. Lethals and semi-lethals in the bovines have been classified by Bane (1964). Lethals are known to affect the bone development other than tissues. Some genes affect several tissues and also produce effect on many parts of the body. The lethals should receive adequate attention in both practical and scientific consideration of animal improvement. It is

and Williams (1930) recorded another case in a Shire mare. This is observed rarely in mares and basically is a bicor-nual pregnancy. As the gestation pro-ceeds the foetus in ventral position rotates and comes to lie beneath the vaginal and uterine body. The dorsum of the uterus becomes ventral and vice-versa, so that the foetal legs point cranially. The rotation may involve a complete turn of  $180^\circ$  which leads to vagina being stretched. In such condi-tion per vaginum examination reveals the elongation of birth passage and the foetus may be palpated below the arm through the narrow elongated vagina. This condition is likely to be mistaken with ectopic pregnancy. Six such cases were observed by Roberts (1956) in mares which had the history of dystokia or retained placenta in previous foalings.

Dystokia in such cases is usually not serious since the foetus does not enter the pelvic canal and the straining is not forceful. At the time of examination usually 24 to 48 hours after the onset of parturition, the foetus becomes emphy-sematous and uterus contracts. The prognosis in such cases is poor. Because of the narrow canal, foetotomy be-comes impracticable and caesarian becomes necessary. It has been shown by Krill and Fincher (cit-ed by Williams, 1943) that bicor-nual pregnancy does occur in cow and possibly in ewe and goat. But this differs to a certain extent from that of mares due to the peculiar anatomical position, the bicor-nual pregnancy in cow

usually results in dorsal presentation. On palpation, dorsum of the foetus is felt crossing the long axis of vagina and cervix at right angles with the foetus lying on its sides.

Diagnosis can be confirmed per vaginum by palpation of withers and ribs. Manipulative correction consists of achieving pelvic version, foetotomy or division of the dorsolumbar articulation. Timely Caesarian should be attempted.

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Malkhede, 1969) Mohanty and Mohanty (1970) reported the occurrence of two bulldog calves in cows presented for difficult parturition. These cows were served by stray bulls and had no history of any bulldog calf in the previous calvings.

## 2. Achondroplasia II or Recessive Achondroplasia

These calves are similar to Achondroplasia I but less severe. They are born alive but die soon after birth possibly on account of respiratory obstruction. The upper jaw is short, so also the leg and the defect is reported to be due to a single recessive autosomal gene. This condition was first reported from Norway in Telemark cattle but similar abnormalities have also been reported in Friesian, Jersey, Guernsey and African Nganda cattle.

## 3 Achondroplasia III

These calves are extremely variable in expression and have a short broad head but the length of limbs is usually not affected. This has been reported in Jerseys and in Swedish Red & White cattle (Bane, 1964) Calves survive longer after birth

## 4. Epitheliogenesis imperfecta or Skinless calves

These defects have been reported in Holstein Friesians Jerseys Brown Swiss, Herefords, Ayrshires, Swedish Red and White and German pied cattle. The skin in such calves is absent on many parts of the body. The defects are usually bilateral and the skin below the knees and hocks and on the muzzle and ears is usually involved. The oral and nasal mucosa occasionally shows the defects. The tongue is smooth due to poor development of horny papillae

which are in turn short and supple. The oral mucosa is tender and such calves are unable to suckle. Salivation is very frequent and when nervous system is involved, the calf is unable to stand. The skin in general has a velvety appearance and skin over the joints is open and these raw surfaces do not show tendency to heal. Such calves are usually born alive but die soon due to bacterial infection. The defect is reported to be due to single recessive autosomal gene.

## 5. Hypotrichosis congenita (Hairless calves)

These calves are born fully developed after a normal gestation period but are devoid of hairs. The hair follicles on microscopic examination show underdevelopment. They are usually born alive but die soon after birth and the defect is due to a single autosomal recessive gene. This has been reported in Japanese cattle, Swedish Red and White and Swedish polled breeds. Streaked hairlessness in Hereford cattle has been recorded by Craft and Blizzard (1934) and Kidwell and Guilbert (1950). Patches of scaly thickened folded skin over the flank and shoulders may be observed. Hairlessness and raw areas may be seen on the joint surfaces. The calves may show extreme salivation but come emaciated and die.

## 6. Aeroteriasis congenita; amputated calves; Hemimella; Amella or Otter calves

These abnormal calves are usually born after a normal pregnancy period and may be still born or die soon after. The degree of manifestation varies greatly and lower portions of the limbs — usually below elbow and hock are absent. The head is abnormal; lower jaw is very short or usually absent.

therefore necessary to prevent the spread and fixation of these undesirable transmissible characteristics. There are limitations to deal with the handling of this problem. Like most other mutations, the majority of lethals are recessive and may remain hidden in a stock for many generations. Test mating and rigid selection help in removal of undesirable characters from a strain. An inbreeding programme designed to eliminate a stock of lethals and sub-lethals also proves beneficial. Close inbreeding is the most certain and quickest means of bringing lethals into lime light. This gives the breeder an opportunity of culling the defective animals. In a herd of cattle, the easier way to get rid of any recessive gene is to test the herd bull before using him extensively. Breeding the bull back to his daughters ensures safety in using him if the character does not show up among the inbred calves.

If the bull carried a recessive, one half of his daughters could be expected to have inherited it. One half of those possessing genes ( $1/4$  of all the daughters) would transmit it to the offspring. Half of this  $1/4$  would receive the gene direct from the sire and also through their dams from the same bull as grand sire. Thus  $1/8$  of the inbred offspring would show a particular character caused by a recessive gene (Gilmore, 1952).

According to Johansson (1961) three methods of progeny testing for lethals can be used.

1. The sharpest test is to mate the bull to known heterozygotes. If the bull produces 11 normal calves and no recessive, the probability that he is a carrier is reduced to about 0.01%.

2. Another test is to mate the bull to daughters of known heterozygotes. If 23 calves of normal phenotype are produced and no defective, the probability

that the bull is a carrier is reduced to less than 0.05%.

3. The third method is to mate the bull to his own daughters, 50% of which would be expected to have received in a single dose of any hidden recessive gene the bull might carry. The accuracy of this test is the same as that of method 2.

Abnormal animals which can be seen to carry and transmit any defect must be discarded, although their progeny in certain instances might be used solely for production. It is not advisable to adopt therapeutic and palliative measures to correct an undesirable factor. Otherwise, the faulty strain will be perpetuated and this will only be a short term policy. Affected animals and their progeny should be culled and eliminated from breeding.

## LETHAL FACTORS

### 1. Achondroplasia I (Dwarf calves or bull-dog calves)

Certain calves of Dexter Kerry breed, Hereford and Friesians, sometimes reveal marked hydrocephalus, chondrodystrophia, concave face and micromelia (Short limbs). Such a calf has a broad compact head with flat depressed nose, prominent lower jaw and the tongue protrudes out of the mouth. The legs and vertebral columns are short and abdomen is distended (Fig. 42, 43). Pituitaries are usually defective and such calves are usually aborted during fourth to seventh month of pregnancy. According to Palianish *et al* (1955), this defect is due to a single autosomal recessive gene with incomplete penetrance.

Achondroplasia in Indian cattle has been reported by Shah (1934), Shanta and Chesh (1936) and Murty (1916). It also occurs in dogs, cats, sheep and goats rarely (Roberts, 1956; Kaikini and

after birth. The condition is considered to be due to a single autosomal recessive gene.

#### 11. Ankylosis of lower Jaw

The lower jaw is ankylosed at the mandibular joint. This has been reported in the Norwegian Lyngdal cattle and is caused by a single autosomal recessive gene.

#### 12. Short spine I or Elk calves

These calves are characterised by reduction in the number and malformation of the vertebrae and ribs. The head and the limbs are normal. Calves are still born or die soon after birth. This has been observed in Osterdal cattle of Norway.

#### 13. Short spine II or Bison Calves

In these calves, cervical vertebrae are reduced to five and thoracic to eleven. The spinous processes of the thoracic vertebrae are prolonged. Hindparts are relatively small. The calves usually are still born. The defect was reported by Zophoniasson (1955) in Icelandic cattle.

#### 14. Ljutikow's Lethal

This defect was seen in Friesian and Brown Swiss in U.S.A. during 1953 to 1956 and was first reported by Ljutikow (1932) in Russian breeds. The foetus is normal in appearance but is either aborted, still-born or dies soon. Gross abnormalities are not present but heart beats are rapid with abnormal heart sounds and occasionally dyspnoea. Death may be due to circulatory failure.

#### 15. Congenital dropsy, Anasarca or water calves

The affected calf is oedematous with accumulation of fluid under the skin, in the muscles, umbilical cord and body cavities. The manifestation of this

condition varies and the weight of calves may be twice the normal. It may be 40 to 100 Kg. and the calf is usually aborted one to two months prior to term or may be born at term with dystokia. The defect occurs in many cattle breeds of the world including Ayreshires, Friesians and Swedish Lowland breeds. The defect is more severe in male calves than the females and the males show higher incidence of this condition.

#### 16. Impacted molars, premolars or parrot mouth

In this condition, the mandibles are short and premolars are impacted. Calves are born alive, but die shortly thereafter since they are unable to suckle. It is seen in short-horn cattle and is due to a single recessive autosomal gene.

#### 17. Atresia and or Imperforate anus: (Fig. 44).

Such calves may live for several days. This was reported by Kuppusswamy (1937) in Ganjam breed and by Kaikini (1963) in Gaolao calves. It is also seen in Holstein, Angus and Guernsey breeds.

#### 18. Atresia ilei

These calves are characterised by complete atresia of the opening of ileum and caecum resulting into great dilatation of ileum. The abdomen is distended simulating ascites. They are usually aborted one to two months prior to term and in most cases cause dystokia. This condition has been recorded in Swedish Polled cattle (Nihleen and Ericksson, 1958). This defect is due to a single recessive autosomal gene.

#### 19. Deformed limbs

Such calves have crooked legs with occasional ankylosis (Fig. 45). They are

upper jaw is curved with sometimes cleft palate and gives the appearance of a parrot bill. At times hydrocephalus may be manifested. The defect has been observed in Friesian and Swedish breeds of cattle and is due to a single recessive autosomal gene.

## 7. Mummification

Mummification has been observed in most of the cattle breeds of the world but its hereditary character is still unclear (Erb and Morrison, 1957). This was first reported by Loje (1930) in Red Danish cattle as carried by a single recessive autosomal gene. Of the two types of mummifications viz. (1) Papyraceous type and (2) Haematinic, the latter is common in bovines. In papyraceous type, the foetal membranes get shrivelled, dried and become parchment like. The fluids are absorbed, uterus contracts on the foetus and moulds on to a dry contorted mass. In the haematinic type, chocolate-like blood mass accumulates between uterus and chorion (including foetus). The anomaly usually arises during the last trimester of pregnancy and the foetuses may be aborted or die at term. They are characterised by a short neck, stiff limbs and occasionally ascites. Hornaday (1917) and Deaton *et al* (1959) considered this to be due to a sex linked lethal gene.

## 8. Hindquarter paralysis

Paralysis of the hind quarter has been reported in certain calves of Red Danish cattle (Loje, 1930) and Norwegian cattle (Tuff, 1948). These calves are usually normal in development and born alive but they are unable to stand on hind legs. The calves are normal at birth but in a month or two dribbling of urine occurs which soils the skin leading to eczema with progressive

weakness of hind limbs. They are unable to stand and since the condition does not improve, slaughter is inevitable. The defect is believed to be due to a single autosomal recessive gene. Neilson (1950) and Christensen and Christensen (1952) demonstrated degenerative changes in the brain and medulla oblongata of the affected calves.

## 9. Muscle contractures or general ankylosis

This defect was reported by Wriedt (1929) in Norwegian Red Polled cattle and by Hutt (1934) in Holstein Friesian, by Stand (1940) and Rogger (1949) in German cattle, by Murray (1951) in New Zealand Friesian cattle. It has also been reported in Red Danish and Jersey breeds and is considered to be due to a single autosomal recessive gene. The calves are characterised by extreme rigidity of neck and limbs and abnormal union (ossification) of one or all limbs. The head is usually drawn backwards and cleft palate may be present.

Limbs are folded under the body leading to dystokia. At times cartilages, capsules and ligaments are also involved causing body ankylosis. A great variation exists regarding the manifestation of the defect. Milder cases, though not lethal, lead to dystokia and embryotomy becomes necessary. The cause is considered to be a hereditary one, although nutritional factors cannot be excluded. A similar condition in sheep is called as 'lethal myodystrophia'.

## 10. Muscle contractures of limbs

A special type of muscle contracture was observed in Norwegian Red Polled cattle by Tuff (1948). Hindlimbs were bent backwards so that the hooves were close to anus. Forelimbs were also flexed and the haircoat was short and frizzy. Calves may be still-born or die soon

## 27 Congenital spasm or hereditary ataxia

This is characterised by intermittent spasmodic movements of head and neck usually in the vertical plane. The calves are born alive but may die shortly after. They are unable to stand and when forced to do so spasms develop in fore and hind limbs. They were reported by Richter and Gehring (1937) in German cattle and by Gregory *et al.* (1944) in Jerseys. This condition is also reported in Shorthorns and Herefords and considered to be due to a single autosomal recessive gene.

## 28 Prolonged Gestation I or Foetal Gigantism

The gestation period may be prolonged in some cows as a result of which the overdue calves are post mature but are normal in appearance and are usually still born. In Holstein Friesian the gestation period may be prolonged by 12 to 98 days (302 to 370 days) in Ayreshires 336 to 381 days in Guernseys by 12 to 246 days and in Japanese cattle 301 to 405 days. Dystokia due to foetal oversize is the outcome of prolonged gestation and embryotomy or caesarian might be necessary. Cows carrying such foetuses show marked deficiency in development and do not exhibit the preparatory changes of parturition. The condition is considered to be due to a single recessive autosomal gene.

## 29 Prolonged Gestation II

The calves are post mature but are abnormal in appearance. The affected calves according to Rasbech (1950-55) showed osteo-chondro-dystrophy, congenital hydrops and hairlessness in Red Danish cattle. In Swedish cattle Hallgren (1951) reported excessive development of the terminal parts of the body

(Acromegaly). The gestation period varied from 332 to 510 days.

## 30 Streaked hairlessness

This is a sex linked lethal character for the male whereas the carrier female exhibits streaked hairlessness in the vertical direction from the back axis. The sex ratio of their offspring shows a marked deficiency of the males. This is considered to be due to a dominant sex linked gene with a recessive lethal action as observed by Eldridge and Atkeson (1953) in Holstein Friesian.

## 31 Congenital Ichthyosis

This condition in calves is characterised by diffused hyperkeratosis and abnormal cornification of the skin which results in the formation of thick horny scales covering the entire surface of the body. The calves are alive at birth but soon die. This has been reported in Norwegian Polled cattle and in Japanese cattle and is considered to be due to a single recessive autosomal gene.

## 32 Adeno hypophyseal aplasia

In these calves the pituitary gland is absent. The affected calves are small usually with short legs and hypotrichosis. In such cases gestation period is usually prolonged up to 500 days and labour which is apparently initiated by death of the foetus is extremely weak. The cows carrying the affected foetuses show little or no development. The condition has been reported in Guernseys by Shorrmont *et al.* (1950) and Kennedy *et al.* (1957).

## 33 Cerebellar hypoplasia/Ataxia

The affected calves are unable to rise after birth but they can be kept alive in bottle feeding. The neck and the limbs are slightly enlarged. Smallness of cerebellum with gross ataxia is one of the

usually still-born but if alive they are unable to stand. The condition has been reported to be due to a single recessive autosomal gene in Holstein Friesian by Rushewsky (1938).

## 20. Brain Hernia

In this anomaly, the sutures of the skull bone fail to close resulting in exposure of the brain. This is also called as cerebral hernia or Caltin Marks. Usually, the frontal and parietal bones are involved and the brain protrudes out. This has been reported in Holstein Friesian. The calves may be still-born or die soon after.

## 21. Brachygnathia inferior or Agnathia-I

This anomaly involves the lower jaw which is markedly shorter than the upper. The calves are born alive but they die soon since they are unable to suckle.

## 22. Agnathia II or Jawlessness

These calves are characterised by the imperfect development or absence of lower jaw. In some cases this may be seen as a wattle like appendage on the sides of the head. Ely et al, (1939) and Lalonde (1910) recorded this condition in Jersey and Ayrshire breeds respectively. The affected foetuses are aborted.

## 23. Sex linked lethal

In this type, the foetuses are expelled dead. Existence of anti male lethal is assumed to be on account of shortage of males. Three types are reported with preponderance of either males or females. Anderson (1910) reported its occurrence in Angler cattle and in Holsteins.

## 24. Fused nostrils

In these calves, the nasal openings are fused and olfactory system is either

poorly developed or rudimentary. Calves may be still-born or die soon after.

Ilancic (1940) considered this defect in Yugoslavian Highland cattle due to dominant gene.

## 25. Missing phalanges; reduced phalanges or creeper calves

In the affected calves, the first and the second phalanges are absent. The metacarpal and metatarsal bones are short. The hooves are usually normal but are connected with the metacarpal and metatarsal bones by only the tendons. The calves may be born alive at full term. The defect is reported by Johansson (1939 and 1942) in Swedish Polled breeds due to a single recessive gene.

## 26. Hydrocephalus

Hydrocephalus involves swelling of cranium due to accumulation of fluid in the ventricular system (Internal hydrocephalus in calves) or between the dura-mater and the brain (External hydrocephalus in pig-lets). The swelling occurs in upward direction and the cranial cavity may be twice as large (Fig. 46). Thinning of the cranial bones occurs due to various reasons such as dietary deficiency, infectious reasons (hog cholera) or genetic factors. The affected calves are aborted or if alive at term show tremors and in-coordinated movements. Sporadic cases of hydrocephalus seen in some breeds may be accompanied by muscle contractures and hydrops amnii, shortening of humerus and femur as in Frisians or congenital hydrops and ear abnormalities as in Ayrshires. Cole and Moore (1942) and Cloninger (1961) considered the defect due to a single autosomal recessive gene. A case of hydrocephalus in a cow-calf was observed by Sane (1965) at Madras which was relieved by caesarian section.

- (xi) Draft Lamb: Lambs born alive but cannot walk due to lesions of the cortical layer of the cerebellum.

### HORSE

- (i) Atresia coli: Closure of colon often associated with brain defects; colts born alive.
- (ii) Frederiksborg lethal: inferred from high degree of sterility in inbred mating of white horses.
- (iii) Sex linked lethal: sex ratio of 2 females: 1 male.
- (iv) Epitheliogenesis imperfecta.
- (v) Deformed forelegs: Atrophied muscles or lack of balance between tendons, characterised by shortening of the phallanges.
- (vi) Hereditary Ataxia: It is invariably lethal and observed in foals of the Oldenberg breed.
- (vii) Hydrocephalus.
- (viii) Erythroblastosis foetalis.

### SWINE

- (i) Brain hernia.
- (ii) Paralysis of hindlimbs.
- (iii) Atresia ani — (Mode of inheritance not clear).
- (iv) Cleft palate — Affected piglings born alive but unable to nurse.
- (v) Thickened forelimbs — Muscle fibres displaced by connective tissue infiltration.
- (vi) Congenital bent legs.
- (vii) Split ears — Associated with deformed hind quarters and sometimes cleft palate.
- (viii) Hydrocephalus — External type.
- (ix) Amputated limbs.
- (x) Diverticulosis or ileitis: Pockets formed by the mucous membrane of ileum associated with marked thickening of the

gut. Irritation caused by the diverticuli inflames the mesentery and local peritoneal adhesions occur.

- (xi) Congenital porphyria (dominant type): Affected animals are characterised by the reddish brown colour of their bones and teeth.
- (xii) Hydrops: Swelling of the nape of neck and abdomen in young pigs.
- (xiii) Clubfoot: Oedematous forelegs and sometimes hind legs as well, inflammation of the lungs and often hyperkeratosis of abdomen and sides.

### POULTRY

- (i) Creeper.
- (ii) Chondro-dystrophy.
- (iii) Amaxilla (Abnormal missing of maxilla).
- (iv) Missing mandible.
- (v) Wingless.
- (vi) Deplochia.
- (vii) Shortlegged.
- (viii) Micromelia.
- (ix) Talpid.
- (x) Crooked neck dwarf.
- (xi) Congenital loco.
- (xii) Skinkiness.
- (xiii) Bilateral microphthalmia.
- (xiv) Blindness.
- (xv) Short upper beak.
- (xvi) Aptylosis.
- (xvii) Short mandible.
- (xviii) Congenital tremor.
- (xix) Naked neck.
- (xx) Short spine.

### DOGS AND CATS

- (i) Stenosis of oesophagus.
- (ii) Cerebellar hypoplasia.
- (iii) Hydrocephalus.
- (iv) Hemophilia.

cortex, is the characteristic feature. Ataxia may be manifested at birth or one to two weeks after. Microscopic lesions may be found in the midbrain and cerebellum. Johnson *et al*, (1958) observed incoordination of synergic muscle in Holstein-Friesian calves due to cerebellar hypoplasia.

### 34. Anodontia with hypotrichosis

Affected calves are completely toothless and hairless at birth. The tongue is longer and thicker than normal. Only three affected males in French cattle of mixed ancestry were observed by Drienx *et al*, (1950) one of which developed two molars and hair after six months.

### 35. Spina bifida

In these calves, the vertebral arches are not fused. The limb muscles are contracted. This may be accompanied by absence of one kidney or atresia ani and brachygnathia inferior. Calves die at birth or shortly thereafter. This has been reported in Norwegian Red cattle by Nes (1959) and is considered to be due to a single dominant gene with incomplete penetrance.

### 36. Partial alopecia and failure of hair growth

These calves are characterised by shortlegs and imperfect development of lower jaw. The calves cannot survive on pasture. The defect was reported in Jaroslav breeds of cattle (Ljutikow, 1934 & 1937).

This defect has also been observed in cattle by Handerson (1955). The affected calves develop stiffness or slobbering at about 2 months of age and which increases with advancing age, terminating in death by about 6 to 12 months.

The following lethal characters have been observed in other species of farm animals.

## SHEEP

- (i) Muscle contracture.
- (ii) Earless and cleft palate often associated with tripartite claws.
- (iii) Paralysis of hind-limbs.
- (iv) Rigid fetlock, Skeleton deformed, associated with short wool and hernia.
- (v) Amputated limbs.
- (vi) Lethal grey. No homozygous grey adults. Grey  $\times$  Grey = 2 grey. 1 Block — Homozygous grey foetuses usually die by 15 weeks of gestation. Affected foetuses exhibit ulcers and hyperaemia of mucous membrane.
- (vii) Dwarfism, Thyroid disturbances leading to death within a month after birth.
- (viii) Congenital photo-sensitivity: Affected animals normal at birth, thrive until they begin to feed on grass. The ears, eyelids and lips first become oedematous, then raw and begin to bleed. Death is inevitable except when the animal is taken inside and sheltered from direct sunlight. Condition is similar to facial eczema in that the surface lesions depend on the presence of phyllo-erythrin. However, the liver is not damaged as in facial eczema.
- (ix) Lethal myo-dystrophia: A muscular dystrophy occurring early in foetal life. Lambs are born alive but die shortly thereafter because of inability to respire.
- (x) Agnathia: Various forms of agnathia.





Fig 42 Achondroplasia

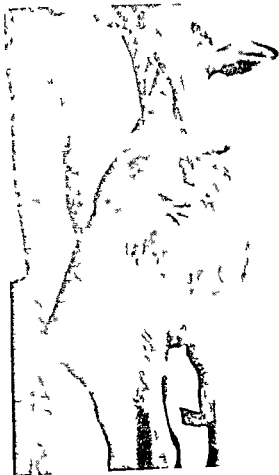


Fig 44 Atresia ani  
(atrectocormus aproctus)



Fig 43 Bull dog foetal monster with one  
eye in the centre



Fig 45 Arched limbs in a  
bull dog foetal monster

# Chapter 12

## Malformations

Malformations in the genital organs are of fundamental importance and are likely to cause infertility and sterility. In recent years, much light has been thrown on a number of aspects of malformations with regards to the types, incidence, aetiology, and the control measures to prevent the spread of malformations in the breeding herds. Sires are being extensively used through Artificial Insemination. It is evident from the records that outstanding sires have been used for a very large number of cows even to the extent of 50,000 or even more. It is necessary to bear in mind, the inherent dangers of transmission of lethal and sub-lethal characters to a large number of offsprings.

Malformations are congenital deformities and are the outcome of an abnormal or arrested development of an organ or of different parts of the body. In all probability, malformations are congenital abnormalities. The word 'Malformation' comprises of mal formashun (maleus-an evil, formatio — a forming, defective or abnormal formation).

Malformation when it involves only an organ or a part of the body, is termed an anomaly. If the deformity is extensive, the individual is spoken of as a monster. The study of anomalies of development is known as teratology.

To study types of malformations, the reproductive system may be divided into three zones.

1. Gonads
2. Tubal zone comprising derivatives from the Wolffian and Mullerian ducts. Wolffian derivatives—Epididymis, Vas deferens, Vesiculae Seminalis. Müllerian derivatives — Fallopain tubes, uterus, cervix, vagina.
3. Copulatory area (penis and clitoris, scrotum, and vagina, vulva).

The mammary system in female is a secondary sexual character closely associated with reproduction and becomes functional soon after parturition.

Tanabe and Almquist (1966) classified the abnormalities as structural and functional with sub-divisions as obstructive and non-obstructive.

Abnormalities may be restricted to a definite organ or region. Malformation characterised as white heifer disease is restricted to the areas formed by Mullerian duct (Spriggs, 1916). In most cases sexual glands undergo hypoplasia affecting both the male and female. Primarily, only the gonads are affected (Eriksson, 1938, 1943; Lagerlof, 1939; Lagerlof and Boyd, 1952; Lagerlof and Settergren, 1952 and Settergren, 1954).



Fig 49 Internal genital organs in a hermaphrodite buffalo



Fig 49 d Ovary testis in swine



Fig 49 b Pseudohermaphroditism in sheep



Fig 49 c Pseudohermaphroditism in mare



Fig. 46. Hydrocephalus in a buffalo calf.



Fig. 47. Cervix bifida in *Bos bubalis*.



Fig. 48. A Kankrej cow with double vagina and duplicate internal organs.

## Incidence

In the absence of authentic information it is difficult to know the incidence from country to country since most of the study pertains to recent years after the introduction of proper records under the sexual health control programme

One of the few systematic studies available in the literature is that of ovarian hypoplasia from Sweden in SkB breed. The incidence was 17.5% in 1936 which declined to 9.4% in 1948 on account of systematic culling.

Tarabe and Almquist (1966) studied incidence, type and severity of malformations of the reproductive system in 180 subfertile and repeat breeding dairy heifers. Gross genital abnormalities preventing conception were found in 27 subfertile dairy heifers. Out of the 27 cases 9 (33%) were congenital abnormalities which included oviducal mesonephric cyst (1), infundibular segmental aplasia (1), oviducal segmental aplasia (1), cornual segmental aplasia (1) and (3) of uterus bicornis bicollis. Out of 1000 cases studied by Perkins *et al.* (1951) cystic ovaries were 2.2%, incomplete tract 0.5%, double cervix 0.3%, uterus unicornis 0.2% and invaginated uterus 0.1%. Kodagali (1968) studied 1058 Indian buffalo genitalia and 172 Surti buffalo genitalia of which 16.5% and 21.4% respectively had genital malformations.

the first pregnancy and in others where the affliction is less the manifestation may not be detected until pubertal age when they become evident.

Genetical analysis by Eriksson (1943) revealed that the malformations of gonads in SkB cattle were due to a recessive autosomal gene with incomplete penetrance. Lagerlof (1962) refers to the morphological defects as an inborn character usually of genetic origin. It is also noticed that there is a correlation between white or light colour and pathological status. White heifer disease is a specific condition in which development of paramesonephric ducts is arrested frequently occurs in white heifers of Dairy Short Horn Breed (Spriggs 1946, Hafs 1946, Finlay 1949, Rendel 1952, Laing 1955, Koch *et al.* 1957). Other breeds having less pigment also seem to be mostly affected by these abnormalities.

Settergren (1954, 1961) showed that ovarian hypoplasia occurs exclusively in cows that are white or almost white in colour. In 6832 cows studied by him 173 had partial hypoplasia and 148 total hypoplasia. All cases with total hypoplasia had 9/10 white colour. It seems likely that the penetrance of the gene for gonadal hypoplasia is dependent on the level of body pigmentation.

Ostrowski (1959) referred to the fact of hereditary diseases affecting fertility



Fig. 49 a. Pseudohermaphroditism in sheep.



Fig 50. Deviated penis in a buffalo bull.  
(Impotentia coeundi).

### 3. Ovarian hypoplasia (incomplete development of ovary) :

The abnormality can be unilateral or bilateral. Ovarian hypoplasia is a morphological defect, a condition in which the whole ovary (total hypoplasia) or a part of the ovary (partial hypoplasia) lacks germ cells. Ericksson (1943) studied the spread of this defect in Swedish cattle and reported that the hypoplasts produced milk with higher butterfat content and this might be the reason for a rapid spread of this condition which reached as high a level as 25.6% in 1935. It was possible to reduce the incidence to 7.9% in 1942 and it was estimated that about 15 more years may be needed to reduce the incidence to 4.5% and a century more to reduce it to the extremely low frequency of 0.5%. This was possible by a nation-wide, vigorous sexual health control scheme and ruthless culling of affected animals. Hignett (1953) reported occurrence of hereditary forms of ovarian hypoplasia in Jamaica Hope and Jamaica Red Cattle.

Settergren (1964) studied the positive association between coat colour and ovarian hypoplasia in the Swedish Highland breed.

Ericksson (1938, 1943) concluded that this condition is caused by recessive autosomal genes with incomplete penetrance (43% in males and 57% in females). Subsequent to this only a single gene is being mentioned (Ericksson, 1949; Lagerlof, 1951). The association between the high butterfat content of milk and this defect is however supposed to be a relationship of physiological nature and not a genetic correlation. Lagerlof and Boyd (1952) in a study of 6286 postmortem examinations in SKB cattle found total hypoplasia twice the incidence in Jamtland (11%) than in other counties (5 to 6.5%). A case of bilateral ovarian hypo-

plasia condition was encountered in a Gir heifer (Kodagali, 1969).

Ericksson (1952) postulated that the gene responsible for this condition probably inhibits secretion of follicular stimulating hormone by the anterior pituitary. Cases involving only unilateral hypoplasia possess normal sexual organs and do exhibit regular sexual activity but have a shorter reproductive life. Out of 8145 cows examined, 13.1% had hypoplasia and of the 1065 cases of hypoplasia 87.1% left sided, 4.3% right sided and the remaining 8.6% were bilateral.

Lagerlof and Settergren (1953) have given a detailed account of this condition in Sweden. A total of 8145 animals of 450 herds were examined between 1931 to 1948. Females were examined from nine months of age and as many times as possible. When the hypoplasia is bilateral the females have the appearance of castrates and lack secondary sexual characteristics. The reproductive organs of such cows are infantile and exhibit a complete lack of sexual activity.

### 4. Cystic ovaries

Henricsson (1965) has carried out investigation of 67 herds comprising 679 SKB cows and reported that cystic ovaries have a genetic predisposition. A gene frequency of 0.7 caused by a recessive gene and a penetrance with increasing age was detected for the dams and higher gene frequency for the sires. Low frequency of manifestation by the younger dams plays an important part in spreading cystic ovaries.

### 5. Fallopian Tubes

Williams (1921) reported blind ends of fallopian tubes. Polding and Lall (1945) reported similar blind ends of fallopian tubes in cattle and buffaloes in

the cause. Some of the malformations may be due to defective gene or as a result of toxic influences and deficiency or surplus of specific nutrients. Experimental exposure of the foetus to such substances has shown to induce malformations (phenocopies), which are normally inherited or acquired.

## MALFORMATIONS IN THE FEMALE

Lagerlof (1956) reported the malformations of genital organs of cows as under:

1. Abnormalities due to developmental defects.
  - (a) Gonadal hypoplasia.
  - (b) Aplasia.
2. Abnormalities due to arrested development of the Mullerian ducts.
  - (a) White heifer disease.
  - (b) Hermaphroditism.
  - (c) Free martins.

Malformations are formed according to three different patterns and they are divided into three main groups (Nes, 1965).

- (i) Bounding of the anterior part of the vagina and its secondary distension with cystic distention of the uterus.
- (ii) Absence of the anterior part of the vagina, cervix and corpus uteri with distended uterus.
- (iii) One cornu uteri with cystic distention, the other cornu being normal.

Teige (1956) summarised information on malformations found in Danish cattle as follows:

(a) Anterior section (Salpinx and uterine horns): Aplasia of salpinx, Uterus duplex (uterus with four horns and four ovaries), Hypoplasia of uterine horns, Constricted passage into one horn, Occlusion of one horn, total aplasia or partial aplasia of single uterus (Uterus unicornis, white heifer disease).

(b) Middle section (corpus uteri, cervix and the anterior vagina): Cervix duplex (uterus didelphys or uterus bipartitus, uterus bicornis bicollis, hypoplasia of cervix, narrow cervical canal, oblique or curved cervical canal, string formation in portio vaginalis and vagina.

(c) Posterior section (vagina and hymenal region): Vagina duplex, Hypoplasia of vagina, Aplasia of vagina, Narrow hymenal ring. Malformation of sinus urogenitalia — Hypoplasia of vulva and vestibulum.

The most common finding is aplasia segmentalis ductus Mulleri. Teige (1956) found 23 cases of this type and the other types of malformations were sporadic.

### 1. Genital hypoplasia

Hamori and Gyuru (1964) reported that four heifers of Hungarian Red spotted breed had hypoplastic genital organs and two more calves were pseudo-hermaphrodites. All affected calves had descended from one grand sire either on the male or the female side. Gordou (1957) reported that six Ayrshire heifers sired by the same bull failed to come on heat at 18 months to 2 years of age and were found to have infantile uteri and non-functioning ovaries. The depression of normal ovarian function was believed to be due to combination of seasonal and hereditary influence.

### 2. Ovarian aplasia (Absence of ovary)

Fincher (1916) reported an apparently hereditary condition of virtual absence of ovaries in three maternal half sisters. These animals showed lack of secondary sex characters and no heat. This condition is reported to be due to autosomal dominant factor. Arthur (1964) reported that in rare instances one or both ovaries may be absent and in these cows the genital tract is infantile and cyclical behaviour is absent.



cellular debris. This characteristic distension of these structures was called "Hydrometrocolpos". Hutt (1946) reported greater frequency of white heifer disease in Short horn heifers of white colour rather than red or roan animals. Fincher and Williams (1926) noticed arrested development of derivatives of Mullerian duct associated with inbreeding in 69% of heifers sired by one bull.

Spriggs (1946) differentiated white heifer disease between three distinct types:

- (i) Group-A—Absence of anterior vagina, cervix, uterine body and a cystic distention of uterine horns. Such animals are always sterile.
- (ii) Group-B—Uterus is unicornis, the abnormality being present as a flat muscular band with a cystic formation near the apex.
- (iii) Group-C—Except for hymenal constriction, the rest of the genitalia is completely normal.

Laing (1955) stated that the following lesions occur either alone or in various combinations:

- (i) A thickened imperforate hymen or a thickened hymen with an extremely small aperture at the site of the normal hymenal vestige just anterior to the external urinary meatus.
- (ii) A completely occluded anterior vagina.
- (iii) Absence of the cervical canal or absence of cervix.
- (iv) Absence of uterine body.
- (v) Absence of one uterine horn.
- (vi) Persistence of prominent Wolffian or Mesonephric remnants.
- (vii) Longitudinal vagina having submucous channels.

The condition has been reported to be associated with white colour in France (Dimitropoulos, 1950). Out of

3315 cows examined, 12 heifers were affected. Of these 10 were white and 2 blue roan. Three of the affected animals were daughters of the same bull and the fourth was his grand daughter. Hanset (1959, a, b; 1960) reported this condition in Belgian White and Blue Pied breed which originated from Shorthorn cattle. Out of 290 cases showing varying forms of abnormality, 237 occurred in white and 53 in blue pied heifers. Hanset (1960, a b) analysed his previous data in addition to data of the same breed in several other provinces and concluded that the hypothesis of an autosomal recessive gene with complete penetrance linked to a partially dominant gene for white 'N', needs revision. It was proposed that the condition has a polyfactorial origin and is not a pleiotropic effect of gene 'N' nor it is due to its allele 'Nw'. It is suggested that the defect might be due to the physiological action of gene 'N' generally in double dose, supplemented by one or more recessive genes at loci independent to the 'N' locus.

Nordlund (1956) reported that 12 (3%) of the daughters of a bull suffered from malformation of the vagina and uterus somewhat similar to white heifer disease which was not seen in the progeny of any other Swedish Freisian bulls and suspected a hereditary bias.

Sebernardori (1959) described cases of pseudohelicoidal uteri in bitches and cats and considered the cause to be hormonal or hereditary.

## 9. Cervix

Cervix duplex condition has been reported in cows by Loen (1961), Settergren and Galloway (1965) and Tanabe and Almquist (1966). Reddy (1959) and Sane *et al.* (1961) observed the malformation in a buffalo cow (Fig. 47). Kodagali and Kerur (1968) in a study

of 1068 Jaffari buffalo genitalia recorded one case each of os duplex and os triplex. Abnormal shapes (Kink and stenosis) were noted in 15 and 6 organs respectively. Bonfort and Mai (1958) discovered the hereditary duplication of os uteri in cattle. Out of 2310 Black Pied Low land cows, 18 had the defect. In another study of 43 affected animals, 20 were offsprings of 5 bulls related to each other. A recessive mode of inheritance has been reported. Sittmann *et al.* (1961) made a genetic analysis of the double cervix condition in cattle in two closely related Hereford cattle and a single autosomal gene with a low penetrance has been incriminated. Sittman (1963) reappraised the previous work and concluded that the double cervix condition in cattle is determined by an autosomal recessive gene with incomplete penetrance.

Van Loen (1963) reported that the relative incidence of this condition was highly significantly greater in daughters of affected dams than those of normal dams. Van Loen (1965) studied the breeding data of 16,375 Meusse-Rhine Yessel cows of an A.I. Association during 1955-1958. The frequency of double cervix condition in cows inseminated was 3.7%. Out of the 132 bulls used, 68 had sired at least one defective daughter. From the 10,181 daughters sired by these bulls, the frequency of double cervix was 1.5%. Reproduction of these cows was normal but they re-

The opening was into the cranial part of the vagina. Cervix was well formed. The other two openings were slightly posterior to os uterus proper. Each communicated independently with uterus by three cervical canals and three external os uteri. The three canals merged into common opening at bifurcation of the uterus. The gonads, horns, uterus, vagina and cervix were well developed. The condition has been reported as being due to failure of Mullerian ducts to fuse into one thus giving rise to triple external os. Three apertures were due to improper fusion of the urogenital sinus. Stenosis of cervix or segmental aplasia of uterine horn is a condition commonly encountered in heifers with mucometra and sterility. Atresia of the cervical canal as a congenital defect has been reported by Paul Cohrs (1967).

#### 10. Vagina

Hull *et al.* (1940) referred to a malformation of tubular genitalia in inbred daughters of one Jersey bull. Vulva and cervix were constricted resulting in difficult parturition following first pregnancies. Surgical intervention in 7 out of 10 cows was required. Congenital hypoplasia of the vagina and congenital atresia of the vagina at the junction of the vestibule in the region of the hymen have been cited by Paul Cohrs (1967).

#### 11. Vulva and Vestibule

proper has been reported in the Swedish cattle by Nordlund (1956). He further reported that the obliteration of the vaginal lumen was not a persistent hymen but as an incomplete recanalisation of the vaginal analage during embryonal life. Hypertrophy of clitoris occurs in cases of functional follicular cysts. Gartner's ducts in the floor of vagina are caudal remnants of the mesonephric ducts which may occasionally persist and become cystic. A case of duplex vagina and vulva in a Kankrej cow was observed by Deshpande *et al*, (1970) (Fig. 48).

## 12. Inter sexuality

Hafez and Jainudeen (1966) have reviewed inter-sexuality in farm animals, which shows that 92% of heifer calves born as co-twin to bull calves are sterile, on account of an abnormal sexual apparatus. They are called as free martins. Lillie (1916) theorised that the direct cause was anastomosis of the blood vessels of the adjacent allantoic sacs. This allowed male hormone from the earlier differentiating foetal testicle to retard the development of undifferentiated gonad and Mullerian duct system of the female co-twin and to stimulate its Wolffian duct system. The result is an intersex, with out-ward appearance of female and internal sex organs of a mixed type. The ovaries remained hypoplastic and occasionally the right ovary descends the inguinal canal and comes to rest on the udder. Vulva shows a prominent tuft of hair in its inferior commissure and clitoris is markedly enlarged. The vagina is typically nonpatent but occasionally it is tubular and ends in the cul-de-sac at the normal position of the cervix. Actually the cervix is absent and the uterus is represented by two solid cords. Abnormal shape and location of exter-

nal genitalia, presence of extremely large clitoris which may be grooved on dorsal aspect diverting the urine upwards and backwards are diagnostic characters. As to the origin of free martin, Bouters and Vanderplasse (1964) stated that the various studies of sex chromatin point to a marked influence of the sex chromosomes on the differentiation of the gonad and the genital tract. The differentiation starts between 30 and 40 days of foetal life when organogenesis of the foetal gonad is not complete. In an individual when cell population or tissues arises with different chromosome complement from a zygote is called mosaicism. In the very individual when the cell populations arise from two zygotes as in twins by placental anastomosis, the condition is called chimerism. Rieck (1963) stated that 8% of female calves born as cotwin to bull calves were fertile. Differentiation can be done by blood grouping and using external anatomical features like bony body, small vulva and clitoris, heavy hair growth. The absence of cervix and the vaginal length are diagnostic. In a calf of 12 to 28 days old, vagina has a length of 12-15 cms and cervix clearly visible. Free martins have vaginal length of 1/3rd of normal length and cervix absent. A speculum of 15 cms long and 1.5 cm diameter is used for the diagnosis. Rieck (1963) observed in free martins as follows:

Age (in days)	Vaginal length (cm)
12-14	4.5
21	4.7
28	5.5

He was of the opinion that a heifer born twin to a bull calf, having a vagi-

Syndrome	Nuclear sex	Gonad tissue	Genital tract	External genitalia	Sex behaviour
True hermaphrodite	Female common (male rare)	Bilateral Unilateral or lateral	Inter-sexual	Female structures predominate	Male-female or inter sex
Pseudo hermaphrodite	a) Male	Testes	Inter-sexual	Female	Male or female
	b) Female	Ovary	Inter-sexual	Male	Female
Free martin	Female	Ovary/or ovotestes	Inter-sexual	Female	Male

nal length of 12 cm or more with a distinct cervix may show normal fertility.

Sterility of the female calf born as a twin to a bull calf was recognised long ago in 28 B.C. (Swett *et al.* 1940). Hunter (1779) reported a free martin condition. Lillie (1917) reported the direct cause as hormone. Moore (1944) referred to this form as under development of Mullerian duct and over development of Wolffian duct. According to him only one in twelve were reported to be fertile. Swett *et al.* (1940) reported 8.3% to be fertile. Joubert (1952) reported that 3.4% of South African Holstein heifers twin to bull calves were fertile.

### 13. Hermaphroditism

This condition is seen in all species of animals but most commonly in goats and pigs (Lisanti, 1936; Asdell, 1942; Yapp, 1947). Asdell (1942) referred to sex ratio in some herds as 55.1 males, 30 females and 14.9 pseudohermaphrodites.

(b) Unilateral — With male and female gonads on one side and single gonad of one sex on other side or a total of three gonads.

(c) Lateral — Male gonad on one side, female gonad on otherside or total of two gonads.

#### Pseudo:

(a) Male pseudo with gonads that are testicular in nature.

(b) Female pseudo with gonads that are ovarian in nature.

True hermaphrodites are extremely rare and have internal genitalia resembling both sexes and external genitalia of intermediate type that may tend towards either male or female depending on structural predominance (Fig. A-19).

True hermaphroditism is probably found in the highest frequency in goats and is a hereditary trait. In goats, the condition is more common in certain breeds like Saanen, Toggenberg, Angora and also certain milch breeds (Cotton 1948; Asdell 1947). This de-

solitary case of hermaphrodites of XY genotype. Kondo (1952) observed 4,629 kids of which 2,371 were males, 2,112 females and 201 were intersexes. When the intersexes were considered as females, the sex ratio was 100:22 compared to 119:28 when they were considered as males. Intersexes in goats must therefore be considered genetical females. The ratio of normal to intersexual kids in litters containing intersexes was 7:1 and of normal female to intersexes 3:1. This conclusively showed that the gene for intersexuality is a recessive autosomal one and only homozygous females exhibit intersexuality. Kondo (1955) further reported that although the non-carriers are usually horned, only two such cases were horned amongst the 41 known male carriers. As against this, amongst the 87 carrier females (that had given birth to at least one intersex) only 3 were horned. Bilgeonre and Buzgunnes (1952) and Brandsch (1959) also reported linkage between dominant Polledness and recessive hermaphroditism in goats. Thus, true hermaphroditism being a hereditary trait, with almost complete linkage to polledness, it can be eliminated by exclusive use of horned breeding stock.

Roslanowski and Lukasik (1957) reported from their studies on a Saanen herd during 1952-56 that the incidence of genital abnormalities, chiefly hermaphroditism averaged 7.6% (19 out of 269). The affected kids were the progeny of seven bucks out of 14 dams. Two bucks and one female produced 7.6 and 4 hermaphrodites respectively.

Attenkirsh and Brandsch (1959) observed 75 litters from matings of polled parents, 44 litters from one polled and one horned parents and 23 litters from matings of horned parent and reported that no intersex ever occurred in the

last mating group. Polled parents produced 1.04 and 7.14% intersexes in two districts. No abnormalities of testicular development or descent were observed in males which were either horned or descendents of at least one horned parent. Brandsch (1959) found that out of 3,693 kids sired by 126 bucks in 1,959 litters, the sex ratio of the progeny of polled parents was 57.68% males; 36.15% females and the remaining 8.15% were intersexes. Out of these kids, only 8.15% of the progeny was horned. Older sires produced significantly more horned offsprings. Though a similar tendency was noticed in females also, it was non significant. There was a linkage for the gene for hornless condition with intersexuality which was expressed only in the genetical females. Eight of the 126 sires were confirmed to be homozygous for polled condition and they produced 9.80% intersexes compared to 4.58% by heterozygous polled sires.

### PSUEDOHERMAPHRODITISM

This condition is common and invariably of male type with testes in abdominal cavity or beneath the skin in scrotal region. Scrotum rarely develops. External genitalia resembles female with enlarged clitoris, vulval configuration and urination in dorsal arc (Fig. 49 a, b, c). Body appearance is like male castrate and fails to show any estrum. Hermaphrodites resemble cryptorchid males or nymphomaniacs in their action and attitude. Yapp (1947) reported cases of pseudohermaphroditism in Brown Swiss bulls.

Recent experimental research has shown that the division of bisexuality (hermaphroditism) into true and false is no longer tenable.

Pseudohermaphroditism is as much true bisexuality as hermaphroditism



and Christensen (1951) have focused attention on problems of male infertility due to malformations in farm animals. Many a malformations are due to heredity and are conditioned by a recessive autosomal gene. The knowledge regarding etiology of some of the malformations is scarce but generally thought to be due to the results of embryonic accidents, genetic mutations, teratogenic effects or hormonal factors. Deficiency syndromes may also be suspected as Vitamin 'A' deficiency in sows which may produce malformed foetuses. In the problems of infertility, it is erroneous to blame only the females since the vital role played by the male in reproduction is also of considerable importance

### Types of abnormalities

#### (a) CRYPTORCHIDISM

Instead of descending through the inguinal canal the testicles may be retained in the abdominal cavity. The condition is occasionally observed in all species of domestic animals.

#### (b) TESTICULAR HYPOPLASIA

Testicular hypoplasia means under development of seminal epithelium and has been reported to occur in some species particularly cattle. The hypoplastic testis is markedly smaller, freely movable and palpable in the scrotal sac. As the hypoplastic testis does not usually sink so far down to the bottom of the scrotum as the normally developed one, the scrotum therefore appears distorted. The consistency varies according to severity. It can be normal, soft or firm. The manifestation of testicular hypoplasia can be one sided, commonly left sided, double sided and partial or total. In bilateral testicular hypoplasia, both the testes are too small in relation to bulls age.

#### (c) SCROTAL AND INGUINAL HERNIA

Williams (1939) reported these defects to be common and economically important, being of hereditary nature. Inbreeding and close line breeding increases the incidence as males with this defect, become physically deficient in serving ability and influence the breeding efficiency. A large scrotal hernia greatly interferes with the testicular function of the affected side on account of the elevated temperature and increased pressure on the testis by herniated intestinal loop. In case of inguinal hernia there is always a danger of strangulation because during coitus the position of the body tends to increase the abdominal pressure and may force the loop of the intestine through the inguinal opening. Warren and Atkeson (1931) were the first to suggest that the condition could be of hereditary origin. Gilmore (1949) observed this condition in Minnesota Agricultural Experiment Station, among animals with close relationship between grand dams and dams but there was no relation between the sires.

#### (d) HEREDITARY SEMINAL DEFECTS

Various seminal defects reported in the literature include acrosomal defects, sperm disintegration, crooked neck spermatozoa, abaxial midpiece, pseudodroplet defect, Dag defect, corkscrew sperm and other abnormalities which are considered to have a hereditary bias.

#### (e) SEGMENTAL APLASIA

Segmental aplasia of the epididymis occurs in all species of farm animals but is more common in the bull, there can be a complete or partial absence of one or both Wolffian ducts. Pioneer work regarding this condition has been reported by Blom and Christensen (1917) in Red Danish bulls. They found a congenital defect in which a portion of epi-

didymis, ductus deferens and seminal vesicles were absent in various degrees. The condition has been described as 'Aplasia segmentalis ductus Wolffii.' The condition can be unilateral or bilateral. In unilateral cases, right side is most commonly affected. They found that right side was involved in seven out of 19 progenies of an A.I. bull in Red Danish Breed. The condition varied from a total aplasia to a marked loss of different segments of the accessory organs. Most commonly the right body and tail of the epididymis were found absent. The result is that due to blind end, ejaculation of semen is affected and when the condition is bilateral there may be total aspermia and sterility. In unilateral cases the fertility may be nearly normal but such bulls should never be used in view of the condition being hereditary in nature.

#### (f) SPERMATOCOELE

It is a cystic dilatation of the epididymis with fluid containing sperms in suspension. Subsequently, rupture of the duct or degeneration of the wall may lead to escape of the spermatozoa which may arouse a tissue response with fibrosis 'Spermatic granuloma'. A case of spermatic granuloma was detected in a Jersey bull by Deshpande *et al*, (1974). Such conditions may be due to obstructions either congenital (aplasia of epididymis) or acquired following injury.

#### (g) SIBACIOUS CYSTIC CONDITION IN THE SCROTAL SKIN

increase in the content of the cyst and also due to the fear of secondary infection and inflammatory reaction surrounding the skin.

#### (h) PERSISTENT PENILE FRENULUM

In this condition, there exists a fibrous band like attachment from the glans penis along the ventral median raphae to the prepuce. The attachment can be cord like and short or a continuous band like tissue (Carrol *et al*, 1964). It is usual that the prepuberal adhesion between penis and prepuce separates completely at about 9 months age. It has been found that there is a hereditary tendency for this condition and has been frequently recorded in Beef Shorthorn, Aberdeen Angus and Polled Beef Breeds, but very seldom in horned beef breeds. Carrol *et al*, (1963) and (1964) in their study for breeding soundness by physical examination and semen evaluation of bulls reported that this type of defect was the third most common penile abnormality. This condition caused severe deviation of erectile penis and prevented normal protrusion and copulation. This can be surgically cured but surgical intervention is not recommended due to suspected hereditary transmission.

Inborn adhesions between the penis and the prepuce are met with in bulls and goats. These are rare and apparently arise from an incomplete separation between the penis and the prepuce.



**(i) CURVED OR DEVIATED PENIS**

It is a condition of abrupt bending of the cranial portion of the penis or glans penis. Therefore, during the erection, the penis instead of taking a longitudinal or normal position, takes a spiral posture in different degrees of severity.

Such a condition prevents copulation or causes difficulty in semen collection with Artificial Vagina. Carrol *et al*, (1963) while examining 10,904 bulls found this condition to be most common cause of abnormality of penis. The configuration can be of corkscrew type. Normally, the heavy fibrous band extends on the dorsal surface of tunica albuginea in the loose connective tissue. In moderate spiral direction this band is in conformity with the normal axis of the penis. But in cases of deviated or spiral penis, during erection it becomes displaced to either side of the terminal portion of the tunica albuginea and thus increases the angle of divergence. Fitzgerald (1963) studied normal and altered anatomy of penis and stated that deviation occurs in those areas where the helicine arteries in the corpus cavernosus penis were poorly developed. Deshpande *et al*, (1967) observed this condition in a Pandharpuri buffalo bull which exhibited normal sex desire but failed to complete the coitus on account of deviated condition of penis even on erection (Fig. 50).

**(j) HERMAPHRODITISM**

The condition is often termed as intersex. There is a combination of both sex characters. The genitalia may resemble to certain degree to male type and also to female type. A true hermaphroditism which is genetically a mixture of male and female is rare in cattle. Pseudohermaphroditism in males are some times encountered. Yapp

(1947) reported such cases in Brown Swiss bulls. These bulls had normally developed secondary sexual characters but were infertile. Histological examination of sex organs revealed absence of functional testicle and duct system. Instead of a normal female reproductive system, a portion of vagina and vulva were present but there was no ovary.

**Diagnosis**

The occurrence of congenital defects of genital organs warrants diagnostic considerations in both male and female, particularly young animals failing to breed. The physical examination of genital organs of all young animals should be carried out prior to purchase. These malformations may cause sterility, infertility, dystokia, abortions and retained placenta. According to Lagerlof and Boyd (1953), ovarian hypoplasia need be distinguished from functional anoestrous condition in heifers. In normal heifers ovaries are large, round, smooth tubular and tract better developed whereas in hypoplasia the ovaries are small, spindle shaped furrowed and the tubular tract is poorly developed. Lagerlof and Settergren (1953) stated that as soon as a heifer is nine months old it is generally possible to determine by rectal examination whether sexual organs are normally developed or not. In case of hypoplasia of one ovary, the rest of the genital system was generally normally developed. The normal ovary which was always on right side could be felt as distinctly larger. It often contained follicles and corpus luteum. The hypoplastic ovary was felt like thin, narrow and rather long structure of a firm consistency. It is felt as a very small cord like thickening. As a general rule, cows with normal ovary and the other totally hypoplastic have normal sexual organs and functions. In

heifers where both ovaries are hypoplastic, the situation is different because the ovaries produce no follicles and there is a lack of oestrogen. Sexual organs remain infantile. There is no occurrence of oestrus nor there is development of female sexual characters. These heifers are like castrates. They have long legs, narrow pelvis, poorly developed udders with small hard teats, uteri small and hard but not malformed. The Veterinarians engaged in sexual health control should take a note of all these forms of disturbances of the sexual organs. Defects of vulva and udder in female are readily apparent.

Diagnosis of malformations is of great importance in selection of sires. In case of diagnosis of malformed sex organs in bulls, it should include the thorough physical examination of sex organs including palpation of testes and epididymis. Rectal exploration will involve examination of seminal vesicles, ampullae and other secondary sex glands. Prepuce and penis should be examined during service and while standing normally. Correct pedigree information and breeding history provides valuable information to trace the hereditary defects. Evaluation of semen samples (macroscopic and microscopic) should also be done.

### Prognosis

Developmental aberrations of genital organs do not constitute a major feature of cattle infertility. Bearing in mind that these abnormalities are hereditary, selection of breeding stock should be done very carefully. Both the anatomical and functional forms of infertility are hereditary. The wide spread use of Artificial Insemination has increased the dangers of dissemination of inheritable abnormalities. It is therefore necessary to pinpoint the animal with such de-

fects and to remove them to check further spread of undesirable genes. Hormone therapy would necessarily prove dangerous as curative measure and is therefore contra-indicated. Surgical intervention or other remedial measures are inadvisable. By such practices sexually inferior animal will be retained for breeding which will perpetuate undesirable hereditary defects. Prognosis is usually hopeless.

### Prevention and Control

Recording of phenotypically defective animals is of primary importance for the control programme. Mason (1964) has detailed out the steps for diagnosis and elimination of genetic defects among farm animals. Only those animals should be used for breeding which have normally developed sex organs. Elaborate precautions are necessary to ensure that Artificial Insemination should be a means of preventing rather than spreading undesirable genes. Regular sexual health examinations have to be done to determine continued freedom from all the malformations in the breeding stock. Culling is the only effective method of prevention. Withdrawal of all malformed animals from breeding programme should be done immediately. Earlier recognition of the malformation is necessary. This will avoid economic loss to the breeders. Lagerlof and Settergren (1953) reported the results of 17 years control of hereditary ovarian hypoplasia by examining all affected animals for breeding. The frequency of ovarian hypoplasia decreased from 17.3% in animals born before 1937 to 7.3% in animals born in 1952-54. Hennricson (1956) suggested that selection should be concentrated on bulls, especially used for artificial insemination. Once the undesirable genes are widely spread out, it is very difficult

and a hard job to eliminate them. If malformations like total hypoplasia spread out widely in future the breed may vanish by itself. Lagerlof (1962) advocated that notoriously cystic cows should not be used for breeding. If treatment is to be used at all, it should be only manual and all hormonal treatment of such animals should be avoided. The control measures adopted in Sweden viz slaughter of affected individual and compensation to the owners was very effective. European association for animal production has suggested a catalogue of hereditary defects to be established and breedwise distribution of the defect studied. Sterility due to causes of anatomical aberrations of the reproductive systems of males and females and endocrine imbalance is probably hereditary. No attempts should be made to correct the anatomical or endocrine causes of sterility lest the condition be spread through a large part of population. Control measures should include survey work by clinical examination in breeding herds (which can be included in routine sexual health control programme) and/or examination of slaughter house material to determine the incidence of different abnormalities. Breed societies should require that all registered animals are free from genital abnormality. A scheme of compensation should be instituted where the incidence is high. A system of checking the incidence of the defects in the progeny of the bulls used for Artificial Insemination should be included.

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# Chapter 13

## Obstetrical Manoeuvres

A veterinarian has to be conversant with several techniques associated in relieving the foetus with as little risk to the dam foetus or the operator as possible

The manipulation by hand has distinct advantages as compared to instrumental manipulation. The hand can palpate the part easily without causing any injury, adopt to the foetal surfaces, perceive resistance offered by foetus and give idea of force to be applied. But in majority of cases, human strength is inadequate and recourse to instruments is necessary. The success with instrumental manipulation depends upon the expert use. Instruments supplement the forces of Nature, rectify presentation or position, diminishing the size of foetus or effecting delivery through *sectio caesarii*.

The obstetrical operations are divided into four major groups

- I Mutation or correction
- II Forced traction after correction
- III Foetotomy
- IV Caesarian section [see Chapter No 56]

### I MUTATIONS — CORRECTIONS

Obstetrical operations which involve change of presentation, position or posture so as to bring the foetus in a nor-

mal presentation, position and posture for expulsion are called as mutations. These include

Complete mutation

- (1) Repulsion or retropulsion
- (2) Rotation
- (3) Version

Partial mutation

- (4) Extension and flexion

The prerequisites for undertaking the mutations are complete dilation of cervix, movements of foetus in the uterus and pelvis, rupture of foetal envelopes and adequate lubrication.

#### 1 Repulsion or Retropulsion

This consists of pushing the foetus forward in the uterus, so as to obtain adequate space for the correction of foetal part in an abnormal presentation. Repulsion is usually carried out in standing position, preferably on a slopy ground with head of the dam downwards. Sometimes repulsion can be easily accomplished but at times it is difficult or impossible. Hand may be used for repulsion, failing this, various instruments may be considered. These include Kuhns crutch, Gunthers repeller and Reindl's repeller. 2 or 3 pronged (Fig 51a b c, d, e). However, the use of such hard instruments increase uterine contractions. These may glide

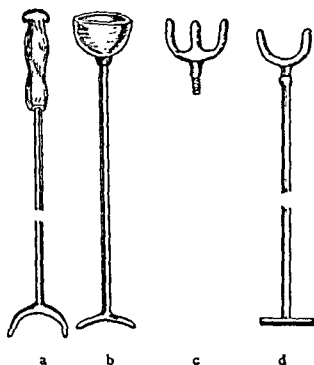
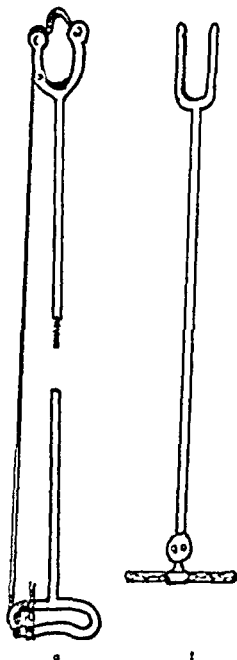


Fig. 51. Repellers.

- (a) Gunther's repeller.  
 (b) Binz's repeller.  
 (c) Reindl's repeller (three pronged).  
 (d) Reindl's repeller (two pronged).

off the slippery surface of the foetus leading to severe injuries to the uterus but are useful when used carefully. It is necessary to secure the part before repulsion. Repulsion can be carried out by hand of the operator or his assistant or by repeller. This should be done after proper lubrication in between the straining bouts by jerks rather than by continuous push.



left and then downwards while the other limb is pulled below the first limb downwards and then obliquely to the right or left causing rotation of the foetal body. In posterior presentation, the foetus is rotated as indicated above with the operator's hand and arm under the foetal buttocks. In milder cases or with small foetuses, the fetlock protruding outside the vulva is flexed and with twisting action, rotation of foetal body is accomplished. In a few cases, rotation of the foetus can be effected by Camerer's torsion fork (Fig. 51f) by fastening the fork to both limbs and rotating the instrument in the desired direction. In ewe and sow, rotation is possible manually whereas in bitch and cat, digital manipulation is effected. Obstetrical forceps can also be used in these species. Rotation is said to be complete when the greatest diameter of foetus coincides with the conjugate diameter of dam's pelvis.

### 3. Version

Version signifies effecting the change in foetal presentation. It is usually limited to 90° and can be effected by repelling one extremity of the foetus and exerting traction on the other. Thus the transverse presentation can be modified to a longitudinal one. Version is done frequently in the mare in transverse presentation but rarely in the cow, bitch and ewe. Version may also be required in some forms of anterior and posterior presentations.

When head of the foetus is moved towards the pelvic inlet, it is called as anterior or cephalic version and when posterior parts of the foetus are moved towards the pelvic inlet, it is known as posterior or pelvic version-podalic version in the human. Pelvic version is usually preferred since it prevents the

head and neck from complicating the correction.

Version includes two important movements — repulsion and evolution. After repelling the foetus into the abdominal cavity, the posterior limbs are seized, corded and pulled towards the pelvis and simultaneously the anterior parts are being repelled towards the cranial end. This is evolution. Reverse holds good in case of cephalic version.

### 4. Extension and flexion

These are partial mutations which consist of extension or flexion of limbs, head and neck that are used for correction of malpostures.

This method involves three basic mechanical principles to effect an easy correction—repulsion of the proximal extremity of the limb, lateral rotation of the middle portion — carpus, tarsus or neck and traction on the distal extremity.

Simultaneous application of these three principles helps in the prompt correction and relief of most of the abnormal postures (Fig. 52 a to h). When the part to be corrected is not accessible, a cord may be pushed around the neck and the two ends twisted outside the vulva, taking care to avoid placenta or cotyledons being entangled in the rope. The twisting of cords facilitates easy access to the part such as neck or limb when correction becomes easier. Sometimes forceps may be used for this purpose. Corrections of hind limbs are more laborious because of the greater length of limbs and movements of the joints being interdependant.

## II. FORCED TRACTION

This signifies withdrawal of foetus from birth canal of the dam by application of force. This is done when the

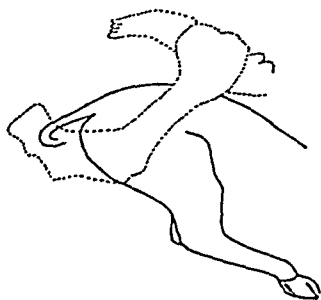


Fig. 52 a. Obstetrical mutations for correction of limb.



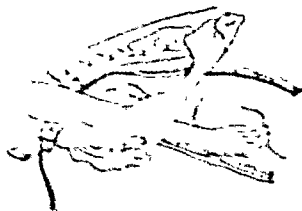
Fig. 52 b.



Fig. 52 c.



Fig. 52 d.



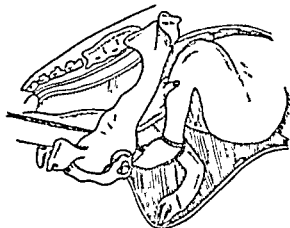


Fig. 52 g. Application of snare on fetlock.

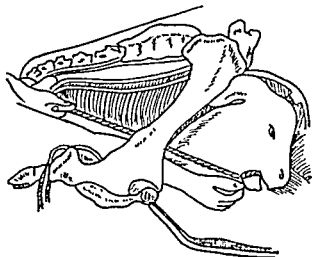


Fig. 52 h. Application of rope on mandible.

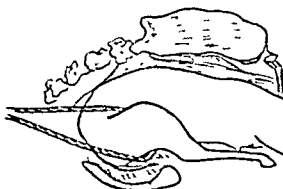


Fig. 53. Forced traction.

expulsive efforts of the dam or operator's attempts are incapable of effecting delivery. Such a force may be developed by cords and bands, crotchets or hooks and forceps (Fig. 53). Lubrication of genitalia is essential for forced traction.

#### Indications

Forced traction is indicated in cases of primary uterine inertia. It is indicated when attempts of the dam are incapable to expel a relatively large foetus. Forced traction can be resorted to in primipara with small birth canal for dilating the passage. It is also applied in narrow birth canal due to certain pathological conditions like tumours, abscesses and fat and also in the posterior presentation to hasten delivery

in order to avoid death of foetus due to asphyxiation subsequent to compression of umbilical cord. Forced traction may be done to save time and to avoid foetotomy under conditions which are not optimum. It can be used as an aid to foetotomy.

In multiparous animals, forced traction with forceps is indicated when only the head of young one has crossed the birth canal and becomes cyanotic.

#### Mechanical means for extraction of the foetus

Use of mechanical means become necessary when efforts on part of the dam or operator are insufficient to deliver the foetus. These may be used to change the position of foetus or to increase the force. Adequate lubrication is necessary.

Instruments and appliances used are grouped as under:

- (i) Cords and bands
- (ii) Pass cords or Portecords.
- (iii) Obstetrical chains.
- (iv) Crotchets or hooks.
- (v) Forceps.

## (i) CORDS AND BANDS

Cords are most useful in Veterinary Obstetrics. They are readily procurable, cheap and portable. Being pliable, they can be pulled in any desired direction.

Cords can be applied to head, limbs or tail. Limbs can be easily seized and offer best support for cording on account of their length and solidarity. The joints prevent slipping of cords. Cords should be applied around the pastern joint in the foal and above fetlock joint in the calves so as to avoid pulling off the hooves. Cords can be applied above hocks and knee joint or lower jaw or even around loins or croup and to foetal body or shreds of skin during foetotomy.

A cord two metres long and 1 to 1.5 cm. in width with detachable wooden handle at one end and loop or iron ring at the other serves the purpose. Nylon ropes two meters long and 1 cm. in diameter are very ideal since they are durable, non absorbant, are easily sterilised and give a firm hold. Cotton ropes once used should be discarded.

During traction, knots at intervals on the cord and wooden handle facilitates traction. Head can be secured by various types of head collars or halters. Jaw can best be corded at interdental space with a simple noose in mouth.

150 cm. in length. Although little costlier, they are durable. They do not absorb moisture and are easy to clean and disinfect. They have good hold but with hard traction may cause even fracture of the foetal bones. Chains cannot be fastened securely around the lower jaw.

## (iv) HOOKS OR CROTCHETS

Obstetrical hooks or crotchets are steel instruments of varying dimensions. These are curved at one end with a ring or an eyelet at the other. The types of hooks required are long, short, blunt, pointed, single or double. The angle of curvature of the hook is important. Wider hooks are difficult to introduce whereas narrow ones slip and cannot be applied properly. With progressive traction, the hook gets anchored in the tissues. The best hooks are Krey

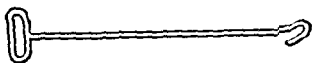
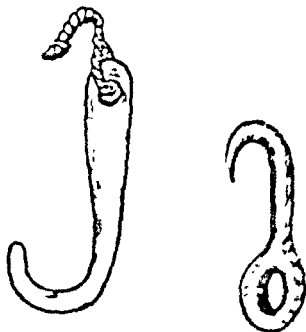
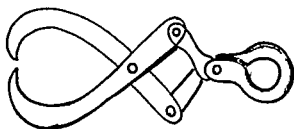


Fig. 54 a. Williams long hook.





d. Krey Schottlers hook.



1. Obermeyers anal hook

Schottler's hook, Robert's hook, Obermeyer's anal hook, William's hook and Harri's hook. (Fig 54 a to e).

The hooks afford a ready means for getting hold on foetal parts where hand cannot reach and the surfaces are slippery. Accidental slipping during traction may result in severe lacerations and injury to the maternal passage. Sharp hooks may be carried inside the tract with pointed end covered by a cork and string — the cork being pulled out at the time of application of hook. Short hooks should always be used with snare.

Hooks may be applied to various parts of foetus, its use depending on the presentation and position of foetus. This is however left to the discretion of the operator. At times, skin is required to be incised before the application of hook. The best location for fixation of hooks are:

- (a) Head: Symphysis mandibulae, palate, orbit, ear-canal, external angle of lower jaw.
- (b) Palatine arch: If the foetus is living, hook applied to palatine arch may render post-natal suckling difficult.
- (c) Spine: Bodies of vertebrae, their transverse processes or ribs.

- (d) Pelvis: Cotyloid cavities, pubis, obturator foramina, base of sacrum and shaft of ilium in posterior presentation.

#### (v) FORCEPS

Forceps have been greatly used in human midwifery. These have not come in general use in large animals because the head of calf or foal is not globular and the forceps therefore cannot be applied with good grip. However, in smaller animals such as bitch, cat, ewe, goat and sow, forceps can be used with advantage. Various patterns are in vogue and include Moellar's De Bruin's, Hobday's, Grey's, Sewell's forceps (Fig. 55). These may be fenestrated, grooved, serrated or toothed. Their blades should be sufficiently long to seize the foetal head and some parts of the foetus. The handles should not overlap each other otherwise, it may pinch the genital mucosa causing great pain. Before applying traction by forceps, it is necessary to ascertain that the foetus is in the pelvis and that no other part of genital tract except the foetal head is included in the forceps.

#### Direction and Degree of traction

Forced extraction of the foetus may be necessary occasionally. However, the degree of force to be applied is dependent upon the presentation, position and period of labour and the number of assistants required. Traction should be simultaneous, without jerks and should be applied during expulsive efforts of the dam. Traction should be continued in an energetic manner, always in a straight line. Time should be allowed for the birth canal to get accustomed to dilate as the foetus progresses during traction.

In small animals light, gentle, steady and sustained traction during expulsive efforts of dam are necessary. Traction

must be applied caudally and downwards in the direction of pelvic axis.

In anterior presentation in uniparous animals, traction is applied on the foetal limbs and head. In standing position, after the head and limbs have cleared the vulva, traction may be applied to limbs alone. Prior to beginning of labour, the major portion of foetus is below pelvic brim and direction of traction should therefore be upwards and backwards so as to bring the foetus in birth canal. When the hooves are at the vulvar orifice, the direction of traction should be upwards and backwards but when the head and limbs have crossed the vulva it should be directed downwards and backwards until it is more or less perpendicular. This direction of traction is in the form of an arc. The abdomen of foetus is relaxed and concave and the back is convex and stretched. The arc shaped traction confirms with the curvature of the foetus thereby preventing hiplock condition or stalling at pelvis. If pulled caudally in straight direction, the linea alba and spine are stretched, foetal pelvis is pulled downwards and forwards leading to hiplock since sacropubic diameter is increased. When the head is near the vulval orifice, upward direction of traction will cause wedging of foetal head in upper commissure and traction should therefore be downwards only. Traction may be given on limbs alternately so as to allow shoulder or pelvis to cross the pelvic brim of the dam. This way, the transverse diameter can be reduced to a certain extent. Traction to the right and left also facilitates passage of the foetus, once the head and limbs have cleared the vulva. Foetal extractors have a limited use.

### III. FOETOTOMY

Foetotomy signifies any obstetrical operation which has the object of reduc-

tion in volume of the foetus either by mutilation or division to be extracted in parts. When the delivery of entire foetus is not possible, foetotomy is performed either in the uterus or when the foetus is engaged in the genital passage. Generally foetotomy is done on dead foetus. The life of the foetus has to be sacrificed.

The actual site of operation may be decided according to the convenience of the operator and the needs. The site should be such which will facilitate easy removal of the excised parts.

Foetotomy may be intra-foetal or extra-foetal. The former method involves subcutaneous removal of foetal parts and is safer since the excised bones or parts always remain covered by foetal skin and therefore do not injure the maternal passage during traction.

#### The advantages of foetotomy

Foetotomy reduces volume of the foetus, requires little assistance, prevents trauma to the dam due to forced traction and replaces Caesarian which is costlier, time consuming, requires more assistance and prompt after care. Caesarian may delay involution of the uterus and result in puerperal complications.

However, there are some disadvantages as it may cause injury or lacerations to birth canal by instruments or sharp edges of bones. It is very heavy and fatiguing. Wounds are likely to be inflicted on the Veterinarians hand from sharp instruments and infected foetus may seriously jeopardise the health of the operator since his hands are likely to get infected. However, the advantages of foetotomy are manifold and therefore it has become a practical and successful proposition in large animals. At least in mares where Caesarian is rarely successful, foetotomy has a predominant role.



If the hand can be passed, a simple wire saw in the form of a loop may be used to excise the young one in sheep and goats but in sow, bitch and cat, foetotomy is not done since Caesarian is usually successful.

Vanderplassche *et al.* (1953) compared the results of 239 foetotomies and 150 Caesarian operations in cattle. Foetotomy was impossible in 3 to 5% cases, 5% cases died, 13% had retention of placenta and 82% conceived within 4 months after operation whereas when Caesarian was done it was impossible in 1% cases, 9.3% died, 39% had retained placenta and 60% only conceived within 4 months. This indicates higher fertility rates after foetotomy than Caesarian operation.

The uterus is closely applied to the foetus and the foetal membranes cling to the fingers during the operation. The surfaces of foetus are slippery on account of foetal fluids. Paralysing, struggling and straining efforts of dam are continuous. The genital tract and mucosa may be swollen possibly on account of previous handling. The operator has to work under all odds and hence obstetrical operations to be undertaken vary according to circumstances and no definite rules can be laid down.

### Indications

Foetotomy is indicated in such cases where parturition cannot be effected by other means without hampering the life of the dam and her subsequent utility. Foetotomy is usually indicated in following conditions:

1. Malpresentations, malpositions and malpostures of the foetus.
2. Relative disproportion between size of the foetus and birth canal.
3. Emphysema.
4. Deformities of maternal pelvis—congenital or acquired, such as

exostoses, fractures, tumours and depression of sacrum.

5. Irreducible distortions of foetus — contractions of muscles, tendons and wryneck.
6. Diseases of foetus — hydrocephalus, ascitis, oedema and anasarca.

### Instruments for Foetotomy

Foetotomy involves the use of surgical instruments for division or puncture. Various instruments have been used which are grouped as under:—

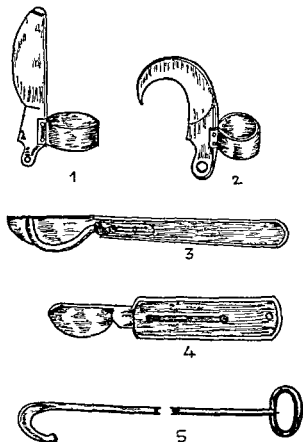


Fig. 56. Embryotomy knives:

1. and 2. Finger embryotomes.
3. Guarded embryotome with sliding guard.
4. Guarded embryotome with spring sliding blade.
5. Long embryotome with cutting edge.

### (i) KNIVES

Knives such as bistoury, Gunther's embryotome, Cole's embryotome and Collin's embryotome may be used (Fig. 56). Difficulty is often experienced to

manipulate them in uterus but these are useful particularly for releasing fluid contents or gas from the foetus. In emphysema, various incisions on skin may reduce the size of foetus.

#### (ii) SPATULA

Spatula may be of use in separating skin in subcutaneous embryotomy. Various patterns are in vogue.

#### (iii) CHISELS

Chisel may be mounted on long handle 75 cm. in length, the blade being 5 × 4 cm. The blade may be curved or straight and used in conjunction with a mallet. These are useful in breaking the foetal bones.

#### (iv) BONE FORCEPS

They are useful but their jaws should be bent and strong.

#### (v) SAWS

Various patterns of saws are available. It may be 40 cm. in length with a blade 10 cm. and are useful in cutting the various parts.

#### (vi) CHAIN SAWS AND ECRASEURS

Surgical chain saw or sector or Persson's parturition saw, Boden wire saw serve the purpose of cutting through shoulder or hip joint or bent neck. The wires can be adjusted to various angles

but are likely to injure the maternal passage.

#### (vii) FOETOTOME

Foetotome is the instrument of choice in foetotomy. Various patterns have been developed — Pflange's foetotome, Arnold's foetotome, Thygeson's foetotome (Fig. 57). The Thygeson's foetotome consists of two steel tubes, 75 cm in length and kept together by means of a stay. It has a head on one side and handle at the other. The tubes enclose the wire saw behind the loop which can be adjusted to varying angles of foetus and does not slip. It cuts through the foetal parts and even the bones. The only disadvantage is that no flap of skin is left over to cover the exposed bones during traction. Since the wire is enclosed in the tubes, it does not injure the maternal passage. The obstetrical wire saw consists of a copper or brass alloy wire 3 metres in length and made up of 8 strands. Handles can be fixed to the ends of wire which may be D shaped, ring shaped, straight or ball formed. The wire can be threaded in the foetotome by means of threader cum brush.

#### Preliminary arrangements for Foetotomy

Once it is decided to carry out foetotomy, epidural anaesthesia may be administered.

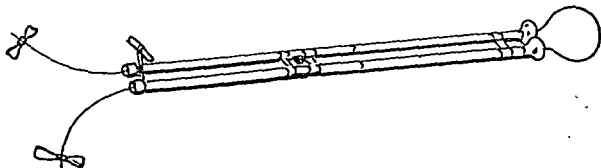


Fig. 57. Thygeson's foetotome.

**Foetotomy operations are as under (Roberts, 1971)**

**A. Operations in anterior presentation:**

Decapitation

Cephalotomy

Amputation of head and neck

" of forelimbs

" of forelimb at shoulder joint

Evisceration

Detruncation

Bisection of Pelvis.

**B. Operations in posterior presentation:**

Foetotomy in normal presentation.

Bisection of pelvis in breech presentation.

Amputation of hind limbs at tarsus.

Evisceration and breaking of ribs.

**C. Other miscellaneous operations:**

Bisecting maternal pelvis.

**Craniotomy, Cephalotomy or comminution of cranium:** This has the object of reducing the size of cranium and is indicated in such conditions as hydrocephalus, deformities of cranium, mon-

strosities, some malpositions and large volume of foetal head.

The operation involves simple puncture, incision or crushing of cranial envelopes (cephalotripsy) or craniotomy, cephalotomy or removal of cranial bones.

The head can be punctured by trocar and canula, scalpel, Cole's embryotomy knife or even by the fingers in some cases. The operation is easy in anterior presentation.

Craniotomy can be done by bistoury, bone chisel or saw.

**Decapitation and decollation:** Decapitation is the separation of head at the atlanto occipital joint. Decollation is the separation of neck at the greatest curvature. These operations are indicated in double headed monstrosities and irreducible malpositions of head and neck. Decapitation and decollation can most efficiently be done by wiresaw or chain-saw (Fig. 58). Thygesons foetotome — the wire being passed behind the atlanto occipital joint or on the flexure of the neck. The wire can be passed

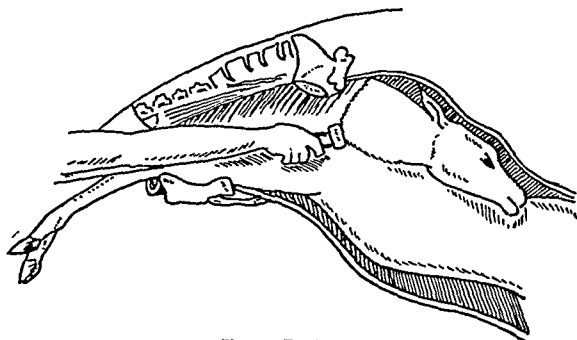


Fig. 58. Decollation.



Fig. 59. Rope carrier.

(a) Schrievers pattern.



(b) Sand pattern.

around, the neck as close to its base as possible with the aid of Schrievers' or Sand pattern rope carriers (Fig. 59 a, b). Head of the foetotome rests in between shoulder joint and neck. The neck and head are then removed by hand or Krey Schottlers hooks.

**Amputation of forelimbs:** Amputation of limbs is often resorted to when they act as obstacles in removal of the foetus. It may be indicated in foetal monstrosities with super-numerary limbs, exaggerated volume of foetus, emphysema, abdominal, hock or thigh presentations and certain malpositions of head and neck.

One or both forelimbs may be removed depending on the situation. It must be borne in mind that limbs afford the best means of traction and should therefore be conserved as far as possible. Skin forms the greatest resistance and therefore subcutaneous method of removal of limbs was developed. (Skellet, 1807; Gunther, 1830; Huvelier, 1830). This method consists of securing the limb above the pastern joint. A circular incision is taken above fetlock or knee

joint on inner or outer aspect. Williams (1943) recommended incision in the scapula on lateral side and extending it all along the lateral length of limbs up to fetlock and then to separate skin by fingers. Skin can be separated by fingers, spatula or pumping in air (Lagarlof, 1969). Traction is applied on the corded limbs when muscles rupture yielding cracking sound or these structures may be removed by fingers and the entire limb from scapular cartilage.

Percutaneous method of removal of fore and hind limbs was advocated by Vanderplasse (1953) and Roberts (1971). This consists of the use of Thygeson's foetotome. Traction is applied on foot by chain or snare. A crescent shaped incision with knife is taken on skin in the area of trapezius and rhomboideous muscles. The dorsal end of scapula is separated by fingers. A wire loop is passed around and head of the foetotome is kept resting on pectoral region (Fig. 60). Alternately,

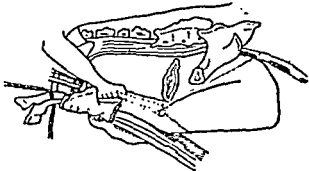


Fig. 60. Amputation of fore limb. Head of foetotome is kept resting on pectoral region.

wire loop may be passed around the limb to be removed and head of the foetotome resting dorsal and caudal to scapula. Apply traction on the limb to keep it tight by chording chain to the handle of foetotome. Acute angled sawing is performed and wire passes through the scapula and the limb is removed. Thus no skin is left to cover

the exposed bones but it is the most easiest and quickest method of foetotomy.

*Amputation of hind limbs:* It may be necessary to amputate the hind limbs in posterior, hock or breech presentation particularly when diameter of foetal pelvis is relatively large and hip-lock has occurred. In hock flexure or monsters like Perosomus elumbis with contracted limbs, a running noose is passed around each leg above joint. Labia are separated and traction applied by 2-3 assistants. Gastrocnemius and lateral ligaments of hock joint are severed. Tibia is pushed in vagina and delivery effected by traction on cords. Foetotome wire may be passed around the hock joint with head of foetotome resting on tarsal bones. In breech presentation various methods have been indicated. One of them consists of taking incision through skin and muscles behind hip joint. Muscles from the proximal third of femur are removed. Femur is corded and the capsular ligament and limb removed by traction. By use of chisel, shaft of ilium and ischio-pubic symphysis are cut through and muscles detached by fingers. Isolated femur is pulled out by traction. Skin is severed below stifle. Other limb is similarly treated. Finally, the body can be removed by traction applied in cotyloid cavity or obturator foramen preferably by a hook. Symphysiotomy was recommended by Carsten Harms cited by Craig (1930). By this operation, the two borders overlap and transverse diameter of pelvis is reduced but its usefulness is doubtful. Removal of hind limb is comparatively difficult than the forelimb since skin adheres very closely, muscles are numerous and powerful and round ligaments offer resistance.

Hind limb can also be removed by subcutaneous foetotomy as in forelimb. The pastern joint is corded, circular incision is taken above the hock and longitudinal incision is carried upto the thigh. Skin is separated by spatula. Gluteal and other muscles are cut across and the limb is torn away by strong and sustained traction.

Foetotomy of hind limb and part of pelvis can easily be done by use of foetotome (Fig. 61, 62). Transverse incision through the skin anterior to external angle of ilium is taken and loop of foetotome wire is passed around and placed in incision whereas the head of foetotome rests between the limbs.

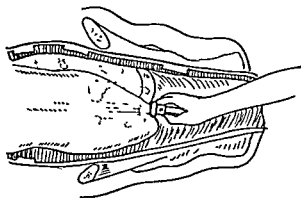


Fig. 61. Amputation of hind limb.

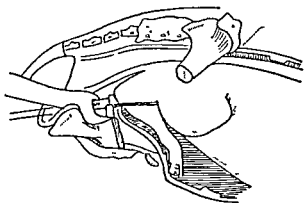


Fig 62. Amputation of hind limb at hock joint.

Alternately, wire may be passed on the lumbar region to include both limbs and head of foetotome resting between the limbs. The lumbar region of the

foetus is cut through cranial to foetal pelvis. This is followed by bisecting the pelvis longitudinally. Benesch (1950) advocated acute angled sawing by keeping head of foetotome on the wings of ilium or even caudal lumbar region. Leg is tightly extended and strapped. A part of foetal pelvis can be included in this. Usually the limb is cut through the hip joint.

Amputation of the limb is not necessary in small animals since they are pliable and do not offer much resistance.

**Detruncation or division of the body:** Detruncation is indicated in certain mal-position or mal-presentations when the foetus is fixed in pelvis and it is impossible to extract or repel the foetus. It may be indicated in dog sitting position, excess volume of foetus, deformity of hind quarters, emphysema (physometra), anasarca and ascitis.

When the foetus is intrauterine, detruncation is a formidable job. However in horizontal dorsal presentation, detruncation may be done as described by Williams (1943). The straining is controlled by epidural anaesthesia and partial narcosis. The foetus is cut through by knife upto vertebrae, which is further passed through the last rib and ilium to cut the flank. Incision is further extended to ventral aspect of vertebrae, the column being divided at the inter-vertebral joint by knife or saw. Hooks and cords may be used for extraction of foetus taking care to avoid injury to the maternal mucosa by ragged ends of vertebrae. At times, it may be necessary to divide spine at more than two parts and even the ribs and sternum.

Detruncation in anterior presentation can also be done by foetotome very easily. The wire loop is passed around the foetal body with head of foetotome

resting on the lumbar region (Fig. 63). After cutting through the trunk, it may be necessary to effect version and remove the foetus by hooks or cords.

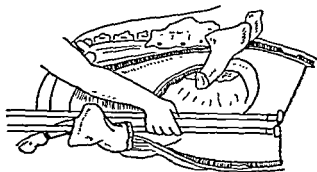


Fig. 63. Detruncation. Head of foetotome resting on lumbar region.

**Evisceration (Eventration):** The volume of thoracic or abdominal cavities may be reduced by removing organs contained within them. It may be done when foetus is emphysematous, ascitic or anasarca. It may be of two types — thoracic evisceration and abdominal evisceration.

**Thoracic evisceration:** This may be done in anterior presentation when thorax is too large. Head and limbs are corded and a knife is placed between first pair of ribs close to the spine and cut downwards along the ribs upto the sternum. First two ribs are cut, through which the lungs and heart are removed. Thoracic cavity then collapses. Hand may be forced further to rupture diaphragm and abdominal organs torn and removed via the thoracic cavity.

Alternately, second to fifth ribs may be removed and so also inter-costal muscles by chisel or hook and organs removed via this vent. Broken ends of ribs are likely to lacerate the genital tract of dam and hands of the operator.

**Abdominal evisceration:** It may be done in anterior presentation as described above. In posterior presentation, eventration can be done by incis-

ing perineum and cutting through sacro-sciatic ligament and removing abdominal organs through this vent.

However, evisceration may be conveniently done by foetotome bisecting the abdominal cavity and removal of organs through this vent.

#### POST-OPERATIVE CARE

After relieving every dystokia case, it is very essential to examine the genital canal for the probable presence of a second foetus. Special care should be taken in case of bitches and sows owing to multiple number of foetuses as they are likely to be retained for several days.

The genital canal should be examined thoroughly for any lacerations, ruptures, invaginated uterine horn, small tears of cervix, vagina and vulva. Use of parenteral and local antibiotics is indicated. If the animal is exhausted, a stimulant draught may be necessary so also some uterine ecbolics such as methargin or ergot extract. In extensive tears of uterus, prognosis is poor and owner should be cautioned. Large vaginal tears should be sutured.

Placenta and placentome should be carefully examined in cattle and buffaloes for presence of any adhesions and

infections. In dog, the retained placenta may be removed by use of a gauze sponge on the forceps.

In most of the obstetrical cases in which forced traction or foetotomy is done, it is advisable to administer 50-100 i.u. of pituitrin for large animals, 20-50 i.u. for ewe and sow and 5-20 i.u. for bitch and cat. This may help to prevent prolapse of uterus, aid in the expulsion of foetal membranes and uterine involution.

Animal should further be examined if it can rise up or whether obturator paralysis, dislocation of hip, pelvic or spinal injuries have occurred.

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PART II  
**GYNAECOLOGY**



# INTRODUCTION

Progress in the knowledge of Obstetrics and Gynaecology is closely linked with the history of Medicine. Credit goes to the Greek Philosopher Aristotle (382-322 B.C.) for bringing about the treatise on reproduction. Ebers papyrus is the oldest book known in human obstetrics and gynaecology published in 1550 B.C. which contains prescriptions of abortifacients to effect labour, treatment for displaced uterus etc. Hippocrates (400 B.C.) has written about menstrual disorders, sterility, symptoms of pregnancy, puerperal diseases etc. He was the 'first' to recommend the use of abortifacients for expulsion of placenta. Soranus in the second century advocated the use of speculum and differentiated the uterus from vagina. Ignaz Philipp Semmelweis (1860) introduced disinfectants for the first time in obstetrical work and proved that parturient mortality could be lowered. For taking care of bacterial infections invading the uterus Lister (1867) further advocated the use of antiseptics.

Albrechtsen (1909), Zschokke (1900), Webster (1932) and others carried out extensive study on sterility in farm

animals. Nielsen (1926) contributed much on researches concerning etiology and pathogenesis of sterility in dairy cows.

Fleming (1877), Williams (1909), Beeman (1931), Benesch (1938), Benesch and Wright (1950) and Roberts (1956) have thrown substantial light, from time to time on the diseases of the reproductive system.

Credit goes to Prof. Nils Lagerlof of Sweden for throwing considerable light on the diseases of the reproductive system and his extensive survey work in most of the leading countries of the world have brought useful information on fertility and infertility in farm animals. He emphasised the importance of periodical sexual health control in consequence of which diseases like, Brucellosis, Tuberculosis, Vibriosis and Trichomoniasis are now under check in a number of countries like Sweden, Denmark, Norway and U.K. As recommended by him, the Indian Council of Agricultural Research, with the aid of various State Governments have implemented sexual health control schemes in

most of the states in India and very useful information is now available on the diseases of the reproductive system in cattle and buffaloes. In addition to the infective factors Prof Lagerlof has enlightened on the reproductive disorders caused due to genetic or hereditary factors such as Gonadal Hypoplasia and various types of congenital malformations. He has also cautioned against the injudicious administration of hormones to combat various types of reproductive disorders. He has specially warned against the risk involved in using sires of low endocrine constitution and has expressed fear that in case such sires are stimulated by hormone treatment, it will result in perpetuating the undesirable characters in the offsprings to come.

In livestock development, particularly with the objective of enhancing milk production, it has become necessary to put every animal under check by periodical examinations to ascertain the health of the reproductive system and the functional status of the ovaries. Attention is also necessary during the various stages of gestation, parturition, expulsion of placenta and much more so on the lochial discharge to differentiate between normal or pathological Puerperal infections have to be controlled in good time and as such a veterinarian has to be conversant with physiology and pathology of reproduction.

Extensive studies on the endocrinology, followed by hormone therapy in various reproductive disorders of non-infectious nature and use of sulphonamides and antibiotics in infective conditions to combat various types of infections, has made a landmark in Gynaec treatment.

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# Chapter 14

## Fertility

### General considerations

In general, the term fertility means to produce abundantly, but in breeding of farm animals, it has a limited sense, since it is limited by the normal physiology of each species, beyond which they cannot normally reproduce. Fertility is a relative term as can be explained that a sow or bitch has a comparatively much bigger litter as compared to the cow and the mare, which normally produce only one young one at a time. Though both are fertile, the number of young ones produced is variable. When we say that an animal is fertile, it is usually meant as the performance of the species in a given environment. The term fertility may be used to express degrees of fertility.

Crew (1924) has defined fertility as the ability of an animal to produce viable offsprings as measured by the rate at which these are produced compared to the standard of absolute fertility, where every ovum liberated is fertilised and where every zygote produced survives. The term infertility is used to express degrees of reduced fertility while the term sterility denotes complete failure of reproductive ability. In general these terms are applied to the female

but in farm animals they are used in the assessment of male efficiency also. In the males, the term fertility is used to express the ability to fertilise and is spoken of as the relative fertilising capacity. The terms infertile and sterile are also used to express reduced fertilising capacity or complete failure to fertilise.

Asdell (1946) defined fertility as a qualitative term denoting only the ability to produce young and the term fecundity as a quantitative term denoting the number of young ones produced. Fertility is reached at puberty but fecundity increases with age and may decrease in later years. In the economics of livestock industry, selection of stock having early maturity, high fertility and high fecundity are highly desirable. The economic performance of an individual animal is also of considerable importance.

Propagation rate in any species is normally dependent on several factors. (1) Average number of young ones produced in each litter (2) Length of the breeding season (3) Duration of gestation and (4) the suckling period, since during high lactation period, heats are suppressed. It is also dependent on the reproductive span in an individual animal which though interlinked with

genetic factors is largely influenced by the environmental factors also.

The average number in a litter in any species is inversely proportional to the average size of the individual of that species. It is well known that greater the expenditure of energy for maintenance, the lesser is the ability to produce new individuals. These are therefore inversely proportional i.e. the individualism and genes vary inversely. There is corresponding increase in fertility where food supply is abundant and environment favourable as a result of which the cost of individualism is much reduced. He has compared the fertility of bats and mice which are much of the same body size. Expenditure of energy in bats is relatively much higher due to their flying habit and as such it affects the number of young ones produced. Similarly it is observed in large animals that wild species have comparatively a smaller litter size than the domesticated ones on a high plane of nutrition such as pigs and sheep. However, this theory can not be applied to express the relative degrees of fertility in all animals

The sexual maturity in males is attained comparatively later than in the female but the reproductive period in the male is comparatively longer than in the female. Under tropical conditions however, the observations are very variable.

It is well known that commonly only one young one is produced in those species in which the period of gestation is longer than six months. It is the usual observation that the larger species of animals have longer gestation periods. The number of teats is also suggestive of the approximate size of the litter. However, this is not true in all species viz., cow, buffalo and mare, though in sow and bitch it holds good. It has

been observed that pigs selected for high fertility may produce a large number of piglets — 16 to 18 — although the highest number of teats varies from 10 to 14. Various reports indicate that even when sheep were selected for higher number of teats, it did not have any bearing on the litter size. The true assessment of fertility is dependent on (1) the number of viable ova shed by the species at each oestrus. (2) the number of ova fertilised. (3) The number of embryos that get chance to develop in a healthy womb throughout the gestation period (4) Normal birth and (5) Favourable or adverse environmental conditions with which the animal has to adjust immediately after its birth.

In addition to these principles the reproductive efficiency of an individual will vary considerably from parturition to parturition owing to the hereditary predisposition and subjective influence of the environmental conditions. Thus, if fertility is normal at a particular stage, it does not necessarily mean that it will remain so during the rest of the reproductive life.

In measuring optimum levels of fertility, one has to consider the genetic make up, the environmental conditions such as soil, water, plane of nutrition, climatic conditions, hygiene and efficient managemental conditions.

There is no accurate formula for measuring fertility but the following factors may give certain indications such as (1) age at puberty and maturity (2) patterns of oestrous cycles and oestral behaviour (3) number of services required per conception (4) efficiency on the part of the male (5) the length of the gestation period (6) normal parturition (7) normal involution of the uterus (8) influence of lactational dioestrus period (9) interval to first post-partum

heat (10) subsequent rhythm of the oestrous cycle (11) service period and (12) intercalving period or calving interval. The term generation interval takes into account all the above aspects but does not take into account the quantitative aspect of fertility i.e. the litter size.

Great variations in the fertility levels have been observed from tract to tract. This is on account of the (1) Prevailing breed type (2) Production potential (3) Exploitation (4) Exposure to infection and (5) Socio-economical conditions of breeders.

Sane (1950) observed that Sindhi and Gir cows reared under climatic conditions of the Deccan plateau in India which are conducive to the breed, were found to have reduced fertility when transferred and reared in heavy rainfall area in Konkan Districts as compared to the local cattle of the tract. Similar is his observation in Murrah buffaloes. Dangi cattle have been found to thrive well in heavy rainfall areas on account of their specialised characters of skin and hooves. Khillar cattle though considered as stronger and faster do not adopt well in heavy rainfall areas with regard to their endurance, draft ability and rate of fertility. Khillar cattle in their home tract under better managerial conditions and an adequate plane of nutrition show good fertility. It was observed that when some stock of young Khillar was transferred from plane to hilly area the heifers could not reach puberty in good

selection. He therefore advises the breeders to avoid selection of such a line of inheritance. It is also a caution to the Veterinarians that whenever they are required to deal with animals of reduced fertility, care should be taken to ascertain that it is not due to the hereditary predisposition. Lagerlof (1951) postulated that fertility of an animal is largely dependent on its endocrine constitution. He is of the opinion that the inherent fitness of the animals to produce is largely predetermined by the hereditary influences, the potentiality is however influenced by limitations of the environmental conditions. As Laing (1954) mentioned that even if a female is very fertile, her fertility is dependent on the chances of the viable spermatozoa moving briskly in the three dimensional space of the reproductive tract and reaching the site of fertilisation in good time as that of a viable ovum. Obstruction or delay on any account will reduce fertility even if the female and the male both are very fertile. Ovulation time is therefore of great importance and so also access of viable spermatozoa. Owing to the limited life of the sperm and the ovum in the intra-uterine tract and the time factor in the process of fertilisation it is generally observed that even in the best fertile herds of cattle, the maximum rate of fertility at first service rarely exceeds 70%. Breeding of cattle and buffaloes by Artificial Insemination, better fertility rates have been claimed on account of deposition of semen in mid-cervix as a result of which the chance

sited in the anterior portion of the vagina and hence the sperm transport takes longer time.

In the mare, sow and bitch even in spite of the fact that the sperms are deposited in the intra-uterine cavity, a fall in the fertility rate is usually observed in case the concentration of spermatozoa decreases. Hammond (1934) has shown that fertility in rabbits is dependent on the presence of large number of spermatozoa at the site of fertilisation to assist in the dispersion of the follicular debris surrounding the ovum. The action of the hyaluronidase present in the spermatozoa facilitates in the penetration of sperms into the ova. Hafez (1962) mentioned the necessary concentration of motile sperms per insemination as follows: Bull — 8 million, Ram 50 to 60 million, Boar — 200 million, Horse — 150 million. Reduction in the concentration may lead to reduced fertility.

Factors which depress gonadotropin production by the hypophysis naturally decrease the number of ova shed. In cases of reduced fertility infectious factors may also be taken into account.

In the economics of livestock industry, the main object is to prolong the useful productive life of a high yielding animal. The production therefore is entirely dependant on high levels of fertility in the reproductive span of an animal's life. Maintenance of good fertility in herds is therefore most essential since the productive health of animals is very closely related to the nutritional needs of the vast human population. During the recent years, attention is therefore being focussed on selection of animals for high fertility and with specific objectives of quality and quantity of production desired from them. Maintenance of good fertility is therefore of paramount importance.

#### Effects of managerial practices on fertility in farm animals

Managerial practices have influence on fertility of farm animals. In natural conditions, the reproductive rhythm is in accordance with genetic make up and environmental condition (Fig. 64). Under domestication, variations are bound to occur right from the selection of seed

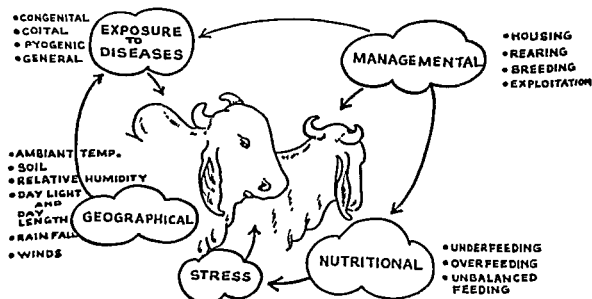


Fig. 64. Environmental factors affecting fertility.

material and its utilisation. In live-stock development, stepping up standards of selection, elimination of sub-standard ones and exploiting the potential capacity of strains having desirable traits are of considerable importance. It is quite likely that if fertility is to be judged on productive capacity, great variations are likely to be encountered. Animals showing higher productive capacity particularly of dairy type are often predisposed to alterations in fertility due to several factors such as stress and strain on the various physiological systems of the body.

When stock is reared on the farm under controlled conditions particularly with the objective of milk production proper housing is the primary requirement.

Housing arrangements made in commercial city stables in Bombay and other towns under Indian conditions where animals remain tied on their standings from calving till they go dry is most unsatisfactory and non-conducive from the point that in turn it reflects on the levels of optimum fertility. Rearing dairy type of stock mostly on ranch conditions and open air enclosures throughout the year as is commonly seen under village conditions in India, reflects adversely on production and fertility.

may cause metabolic stress affecting fertility. Likewise, low levels of specific nutrients over prolonged periods may affect fertility. In managerial conditions, available water facilities are of considerable importance and ultimately go a long way in the maintenance of fertility.

Hygienic conditions have marked influence on levels of fertility. Unhygienic conditions may predispose the stock to several infections affecting fertility.

Extreme climatic conditions are not conducive to normal fertility.

Under controlled breeding conditions several errors in the managerial practices are likely to occur in mating the animals in good time in consequence of which even the best fertile ones may remain without service. Detection of heat is a problem in big herds particularly when the animals are left on the pasture without a bull or a teaser. Thus, in a number of animals, the heat periods pass off without detection and service.

Method of breeding by Artificial Insemination has created a number of problems since it is very difficult to carry out inseminations in good time close to ovulation period. This reflects on levels of fertility. The viability of sperm is another factor in breeding by artificial

semination though the quality of semen from reliable stock is known for certain, cent per cent conceptions is not the rule. Alterations may occur owing to several factors, which lead to variations in the degrees of fertility. Inseminations close to ovulation time may show a higher degree of fertility. This is therefore entirely dependent on the knowledge and skill of the technician. Farmers experience to detect heats in females and report them for inseminations in good time is also an important factor in the maintenance of good fertility.

A good deal of importance is attached to homebred stock. The year in which replacement goes difficult on account of less number of heifers coming into the productive age, the farmer is forced to purchase animals from outside sources for keeping the numerical strength of the herd. There is inherent danger involved in such a type of replacement from outside sources, since the incoming animals are likely to be exposed to infections already present in the herd and become involved to the same causative factors. It is also likely that the newly introduced animals may bring along with them known and unknown types of infections to which the animals in the herd will be exposed. Both the above factors are likely to affect fertility.

It is possible to diagnose pathological conditions in the newly introduced animals in the herd. However, genetic factors viz. lethals and sublethals cannot

be detected as these effects are expressed after a number of generations.

Hereditary characteristics are closely associated with health of animals. These genetic factors are largely intrinsic in nature, some of them are predisposing and others activating. The fertility of an animal is mainly dependant on genetic potentialities. To some extent fertility is also dependent upon how a foetus gets an opportunity to develop in a healthy womb. Two important factors namely, that tissues are forming and are being nourished in a disease free womb during gestation period also influence the health of the foetus. It can therefore be said that the inherent constitution and prenatal life possibly govern the potential breeding efficiency of an individual and the way it will react to favourable and unfavourable conditions.

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# Chapter 15

## Hormones of Reproduction in the Female

In female farm animals growth and reproduction are vital processes governed by hormones. Hormones are chemical substances synthesized and secreted by ductless glands and carried to target organs via the blood stream for producing their effects. The word hormone as defined by Bayliss and Starling (1902) included substances that influence an existing reaction where as the local hormones or para hormones, viz. prostaglandins, erythropoietin and histamines are produced locally acting on surrounding tissues. The neuroendocrine constitution of the individual depends on the

genetic make up and its interaction with environment.

Chemically the hormones can be classified as: (i) Protein or Glyco-protein hormones e.g. gonadotrophins and (ii) Steroid hormones e.g. gonadal hormones. In addition, there are releasing hormones or factors originating in the hypothalamous which control release of pituitary hormones. The reproductive hormones including hypothalamous releasing hormones, hypophyseal hormones and the gonadal hormones.

The origin, site of action and their effects have been presented in Table 19

Name of Hormone	Origin	Site of action	Functions
<b>B. HYPOPHYSEAL HORMONES</b>			
1. FSH Follicle Stimulating Hormone	Anterior pituitary	Ovary, testis Seminiferous tubules	Follicular growth, stimulation of 'Sertoli cells', spermiogenesis
2. LH Luteinising	Anterior pituitary	Ovary, testis Leydig cells	Ovulation, CL formation, secretion of progesterone oestrogen & androgen
3. Prolactin	Anterior pituitary	Mammary gland, male organs	Lactation, progesterone secretion, testosterone secretion, stimulation of male accessory organs.
4. Oxytocin	Posterior pituitary	Mammary myoepithelium, uterine myometrium	Parturition, milk let down, sperm and ovum transport.
5. PMSG (i) Pregnant mare serum gonadotrophin	Placenta	—	Predominantly FSH like and LH like in action.
(ii) Placental luteotrophin	Placenta	—	Maintenance of pregnancy CL
<b>C. GONADAL STEROID HORMONES</b>			
1. Oestrogens	Ovary placenta	—	Maintenance of secondary sexual characters, growth of female reproductive tract. Feed back mechanism of gonadotrophins, oestral behaviour, promoting duct growth in mammary gland, increases uterine contractility anabolic effect.
2. Progesterone	Ovary	—	Synergistic action along with oestrogen for oestral behaviour, promotes proliferation of endometrial glands, alveolar growth in mammary gland, negative feed back mechanism on gonadotrophins, maintenance of pregnancy, inhibiting uterine contractibility.
3. Relaxin	Ovary (CL)	—	Relaxation of pelvic ligaments and symphysis pubis.
4. Androgens	Testis	—	Maintenance of secondary sexual characters and accessory sexual organs. Stimulates spermatogenesis, Libido, male behaviour, anabolic effect.

Role of Hormones during various phases of Reproduction:—

### **Growth and Puberty**

The body growth is influenced mainly under the Somatotrophic Hormone (STH). When the body growth is fully attained gonadotrophic hormones come into play in initiating the growth of the genitalia. Interactions of various hormones are responsible for regulating the reproductive processes.

In the female, hormonal activity responsible for the occurrence of the first oestrus is similar to that responsible for the subsequent oestruses. In prepubertal animals, probably the gonadotrophic levels in the pituitary are high but they lack the ability to release. Before puberty is reached it appears that the anterior pituitary gland must first start secreting or releasing sufficient amount of gonadotropins into the circulation to stimulate production of oestrogens by the ovary.

### **Oestrus and oestrous cycle**

The various events which occur during the oestrous cycle are controlled by several pituitary and interrelated ovarian hormones. FSH stimulates growth of the Graafian follicle and is further responsible for maturation of the ovum. LH initiates the enlargement of the pre-ovulatory follicle, resulting in ovulation and formation of CL. FSH & LH synergistic

sible for rupture of the follicle and release of the ovum. After ovulation the levels of oestrogen and gonadotropins are greatly reduced. The CL which is formed after ovulation, starts secreting Progesterone. LH and Prolactin have a synergistic effect in activating progesterone secretion by the CL. Progesterone exerts a negative feed back on the hypothalamous and pituitary decreasing the levels of gonadotropins. As long as the CL is functioning, production of progesterone is high, the FSH secretion remains low and there is little follicular activity. If pregnancy does not follow then production of progesterone by the CL falls down, thereby increasing the production of FSH and resulting in growth of fresh crop of follicles. Thus it appears that high level of progesterone suppresses the FSH secretion. At oestrus the concentration of LH in the circulation may increase 200 to 300 fold. The concentration of FSH hardly doubles at this time (Niswender *et al*, 1974).

### **Pregnancy**

Niswender *et al* 1974 reported that in the early part of pregnancy, there are no endocrinal changes. On conception a signal from the embryo prevents the regression of corpus luteum by possibly preventing production of luteolytic factors. They further suggested that the embryo may produce luteotrophic substances which may neutralise the luteo-

After nidation, there is gradual increase of progesterone in the circulation. This continues until few days prior to parturition when oestrogen level (placental origin) rises suddenly. The role of ACTH during pregnancy is not clearly understood, however it is concerned with metabolic processes essential for normal pregnancy.

In equines, the source of progesterone is mainly from CL and accessory CL. During pregnancy the endometrial cups (at the stage of 30 to 170 days, with a peak at 60 days) secrete large quantities of PMSG which stimulates follicular growth resulting in ovulations. These lead to formation of accessory CL.

### PARTURITION

Considerable variations in the levels of circulating hormones occur a few days prior to parturition and subsequently at parturition. The progesterone level suddenly drops, which overcomes the progesterone block maintained during pregnancy. This results in the rise of oestrogen level which sensitizes the myometrium. At this juncture, oxytocin comes

into play and in conjunction with oestrogens causes uterine contractions. The peak level of Oxytocin is reached at parturition. There is also a rise of ACTH just prior to parturition but its role is not still clear.

Relaxin causes relaxation of pelvic ligaments at parturition there by widening the birth canal.

In the initiation of parturition gonadotrophins from foetal pituitary may act on placenta for production of oestrogens (Foster *et al*, 1972). So also the foetal corticosteroids may initiate parturition.

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# Chapter 16

## Puberty and Sexual Maturity in the Female

Puberty is the stage at which various systems of the body have reached their optimum development of functional ability. In the female the reproductive system starts functioning with expression of the first signs of oestrus followed by establishment of regular oestrous cycles in the female.

Expression of the first oestrus does not necessarily mean that the animal has reached sexual maturity.

Reproduction in the female is a complicated phenomenon dependent on various factors. Efficient reproduction is largely dependent on the hereditary characters, endocrine constitution, intra-uterine development during foetal life, weight at birth, environmental conditions, nutritional status during growth period and managemental conditions. It is also dependent upon how the foetus gets an opportunity to develop in a disease-free womb.

If the physiological processes are in harmony and the various factors described above are conducive, the female will reach puberty in proper time. The age of attainment of puberty, is however dependent on the species.

Puberty can be considered as a vital period in the life of a female when gross

changes occur owing to setting up of the functional activity of the reproductive system. It is usually considered that when regular ovarian function is established the female is said to have reached puberty. The visible expression is by way of exhibition of secondary sexual characteristics. The onset of puberty is a gradual process. During physical growth period there is hardly any development of the reproductive system and it is only after full attainment of growth of the various physiological systems, that the nutrients are diverted to the development of the gonads and reproductive tract. No functional activity is expressed by the reproductive system before puberty. It has been observed that the body weight is correlated to the development of the reproductive organs. During growth period the body is under the influence of the somatotrophic hormone (STH) and when optimum level of growth is reached, the level of the somatotrophic hormone drops and in turn the anterior pituitary starts liberating the follicular stimulating hormone (FSH) which causes development of graafian follicles in the ovary. Probably certain neural stimuli cause the hypothalamus to produce or release the factors which in turn cause the release of the gonadotrophins into the

blood stream at puberty (Lasley, 1968). The developing graafian follicle contains oestrogen a hormone secreted by the ovary which brings about growth of the various parts of the tubular genitalia. An optimum level of oestrogenic hormone induces the animal to oestrus and makes it receptive to the male. It is due to the manifestation of oestrus, one can make out that the animal has reached puberty or sexual maturity. Exhibition of the first oestrus does not necessarily mean that the body has reached full growth and sexual maturity including regular breeding efficiency. It is usually observed that the first few heats may be unovulatory with irregular cycles. In certain well fed animals oestrus is exhibited early in life when body growth is still to reach its optimum level. When normal regular cycles are established the animal is said to have reached sexual maturity.

#### Age and body weight at puberty

Species-wise differences are noticed with regard to the age and body weight at puberty. Age of the animal is not the criterion but relative growth and body weight influence the onset of puberty. The period of puberty also varies in different breeds and is closely associated with the nutritional status.

The average age at puberty in exotic (*Bos taurus*) cows ranges between 8 to 18 months and 24 to 30 months in Indian cows of well defined breeds. In non-descript cows this period may extend over 36 to 48 months. Buffaloes under Indian conditions reach puberty on an average between 24 to 30 months, depending on the breed and environmental conditions. Such a wide variation is noticeable on account of diversity of environmental conditions, and husbandry practices.

Mares reach puberty at about 18 to 24 months. Even though puberty is reached at this age breeding is always delayed until three to four years to allow optimum growth.

In ewes, puberty is reached by 8 to 12 months.

Goats attain puberty at an average age of 6 to 18 months.

Sows attain puberty on an average between 6 to 12 months.

In bitches, the onset of puberty varies to a great extent depending upon the breed and domestication and usually occurs between 6 to 12 months.

Cats reach puberty between 6 to 15 months.

Hens start laying eggs on an average at the age of six months.

#### Puberty in Cows

Ahuja *et al.* (1961) reported that the age of puberty in Haryana heifers is 919 days. Sane *et al.* (1966) recorded the age at puberty in Nimar cows as  $1344.9 \pm 48.6$  days. Rajgopalan (1952) recorded the mean age at first heat in Sindhi heifers as  $1030.8 \pm 35.3$  days and  $1114.75 \pm 44.9$  days in Kangayam breed. Kumaran (1951) recorded the mean age at first heat in Tharparkar breed as 683 days (range of 427 to 988 days) and in Sahiwal breed as 677 days (range 427 to 954 days). Luktuke (1963) reported an average age of puberty as 1067.7 days with earliest heat at 919.7 days in Haryana cattle. Sane *et al.* (1968) reported the mean age at first heat in red Sindhi as  $957.5 \pm 18.34$  days and  $1181.0 \pm 18.20$  days in the Gir breed. The average age at first fertile heat in Gir cows was  $1200.00 \pm 48.74$  days. Purbey (1965) reported age at first heat and at first fertile heat in Dangi cows

as  $1107.5 \pm 42.02$  and  $1154.0 \pm 35.11$  days. Tatke (1967) reported the respective values in Khillar cattle as  $1026.75 \pm 21.82$  and  $1049.36 \pm 21.84$  days. Ponkshe (1969) reported these values in Gir as  $858.72 \pm 29.12$  and  $1007.95 \pm 22.56$  days respectively. Dange (1969) reported in another Gir herd the respective values as  $1329.66 \pm 41.78$  and  $1361.66 \pm 43.66$  days. Sonawane (1969) reported the mean age at first fertile heat in Rathi heifers as 715.56 days. Velhankar (1973) reported the average age at puberty in Gir heifers as  $911.31 \pm 75.75$  days, the corresponding weight being  $271.18 \pm 26.11$  Kg. The correlation coefficient between age and weight at puberty was high ( $r = +0.5475$ ). (Table 20).

Hammond (1927) reported the average age at puberty for all the continental breeds thus: (range 5 to 15 months), Jersey 8 months, Guernsey-11 months, Friesian-11 months and Ayrshire-13 months.

### Puberty in Buffaloes

In general it has been reported from Bulgaria, Malayasia, Cambodia and India that the average pubertal age is reached at about 3 years (Kalef 1932, Marsh and Dawson 1948, Baradat 1949 and Bhattacharya 1953). Hafez (1955) reported that the average age at first oestrus in buffalo heifers is 405.5 days and the average age at first conception is 647 days. Knapp (1956) stated that in Egypt the buffalo-heifers are usually bred at the age of two to two and half years. Gorbali (1935) reported that in Azerbaijan the buffalo reaches puberty at the age of two to three years and with good feeding even at a year and half. Sane et al (1968) reported that in Murrah and Surti buffaloes the mean age at first fertile heat is  $1089.69 \pm 114.32$  and

$940.0 \pm 30.24$  days respectively. (Table 21).

### Puberty in Ewes

Terrill (1968) stated that the first oestrus in ewes may occur at 4 to 10 months of age with live weight of 40 to 60% of mature weights. Hafez (1952) reported that quite a number of ewes do not have even one oestrus until in their second year. Unovulatory heats generally precede the first oestrus. Hampshire and Rambouillet ewes normally reach sexual maturity at one year. In seasonal breeders, the onset of puberty is affected to a certain extent. Early born lambs of a seasonal breeder may reach puberty at 6 to 8 months of age while as the late born lambs in season may reach puberty at about 14 months of age (McDonald 1969).

Under Indian conditions, the ewes reach sexual maturity from 9 to 14 months of age, however, the full body growth is only attained between 18 to 24 months of age the stage at which mating is preferred (Anon 1962). Merino sheep mature more slowly than the Hampshire or Suffolk breeds. Cross bred lambs generally mature earlier than purebred lambs.

Roy et al (1962) recorded first oestrus in young Bikaneri ewes of less than one year of age.

### Puberty in Goats

Puberty in goats occurs at the age of 249 to 293 days. Sexual maturity varies with the breed and rate of growth. Most goats are bred after they reach one year of age (Raja, 1977). Lall (1954) reported puberty age in Beetal does as one year three months. He further reported that the pubertal age may be as early as six months but the mating should be de-

Table 20  
AGE AT FIRST OESTRUS IN COWS

Breed	Age at first Oestrus (in days)		Reference
	<i>Average</i>	<i>Range</i>	
Haryana	1177 50 ± 20 98	—	Choudhary <i>et al</i> (1965)
Haryana	900 00	600—1170	Sharma <i>et al</i> (1968)
Haryana	1410 00	—	Singh <i>et al</i> (1968)
Gir	911 32 ± 75 75	—	Velhankar (1973)
<i>First fertile heat</i>			
Red Sindhi	840 00	—	Burkule (1969) Burkule (1969) Velhankar (1973)
Sahiwal	840 00	—	
Rathi	756 78	—	
Gir	926 00	—	
Gir	978 66 ± 28 73	—	
Thari	870 00	—	Burkule (1969) Sanc (1975) Sanc (1975)
Haryana × Jersey F <sub>1</sub>	679 33	—	
Karni Swiss	615 00	—	
Red Sindhi × Brown Swiss F <sub>1</sub>	615 00	—	
Rathi × Jersey F <sub>1</sub>	614 17	—	
Rathi × Jersey F <sub>1</sub>	531 1	—	
Gir × Jersey F <sub>1</sub>	543 25	—	
Gir × Holstein Friesian F <sub>1</sub>	561 00	—	
Gir × Jersey F <sub>1</sub>	510	—	
Rathi × Holstein Friesian F <sub>1</sub>	788 37	—	
Rathi × Sahiwal F <sub>1</sub>	814 ± 15	—	Sanc (1975)
Rathi × Sindhi F <sub>1</sub>	901 32	—	
Rathi × Gir F <sub>1</sub>	868 33 ± 175 14	—	
Gir × Sahiwal F <sub>1</sub>	990 ± 68 56	—	
Haryana × Holstein Friesian F <sub>1</sub>	714 62	—	



Table 21  
AGE AT FIRST CALVING IN BUFFALOES

Breed	Age at first Calving (in months)		Reference
	<i>Average</i>	<i>Range</i>	
Murrha	43.4	—	Agarwala (1952)
Murrha	44.3	—	Venkayya and Anantkrishnan (1957)
Murrha	42.31	—	Gautam and Vimal (1966)
Murrha	40.6	—	Arya and Desai (1969)
Murrha and Nili	—	37 to 40	Amble <i>et al</i> (1970)
Bhadawari	50.7	—	Singh and Desai (1962)
Egyptian	38.0	22 to 56	Khisin (1951)
Egyptian	38.68	22 to 60	Sidky (1953)
Egyptian	39.9	—	El Sheikh (1967)
Phillipine (Carabao)	43.6	38.6 to 51.5	Ocampo (1939)
Phillipine (Carabao)	38.3	33.4 to 43.6	Villegas (1959)

layed until the doe attains one year. The age at first kidding reported by him is one year eight months to one year ten months. Amble *et al* (1964) reported the average age at first kidding in Beetal goats ranging from 745 days to 775 days. Raja and Mukundan (1963) reported the average age at first kidding in the Malabari and Jamnapari-Malabari crossbred goats as  $495.05 \pm 18.86$  days and  $533.90 \pm 20.26$  days respectively and the kidding interval reported was  $284.60 \pm 18.15$  days and  $299.27 \pm 16.17$  days respectively. Rate of kidding was higher in winter than summer and rainy months in both the breeds. Season did not exert any significant influence on the length of kidding interval. Sacker and Trail (1966) reported the mean age at first kidding in

Mubende goats of East Africa as  $567 \pm 11.6$  days.

### Puberty in Mares

In general, the fillies attain puberty between 15 to 18 months. With definite breeding season in certain areas, sexual maturity in mares is reached in spring or summer at the age of 25 to 28 months (Nishikawa & Hafez, 1968). It is observed that after 2 to 3 oestrous cycles, the sexual behaviour of the fillies is similar to that of mares.

### Puberty in Swine

Sexual functions in sows begin at 4 months of age with periodic sexual interest but the immobilisation reflex and ovulation do not occur at this age

(Smith 1968) First true ovulatory oestrus does occur before 5.6 months of age, although considerable variations are observed. Females not expressing oestrus before 8 months of age are considered by Smith (1968) as cases of delayed puberty. Du Mesnil Du Buisson *et al* (1970) reported the average age at puberty as 7.8 months.

The species specific characteristics of sows puberty is that in case of large group of gilts which are nearing puberty, certain stress effects as well as the introduction of boars will produce a well synchronised oestrus within

few days. Such stress effects might be caused by transportation transfer or regrouping of gilts which are close to puberty. The male effect is more evident when the gilts are kept in close proximity of boar after their transportation — oestrus with sharp peak being expressed within 4.6 days in 26% of cases. If the boars are introduced 10 days later after the arrival of gilts oestrus with sharp peak is expressed after 26 days [Du Mesnil Du Buisson *et al* (1970)].

Puberty in swine is primarily connected with age and not the body weight. Even in case of intensive rearing where ad lib feeding is available from self feeders, puberty will be delayed on an average by 14 days. The time of first mating is also determined on the age and body weight. Becze (1961) after following these criteria at first mating observed that 20% of gilts had infantile genitalia and that their number decreased with increasing age.

In gilts when full development of genitalia is not attained there will be reduced ovum production below breed average, resulting into small litter size. This is evident in first maturing hybrid pigs since they gain faster body

weight which is considered appropriate for maturing strains having higher levels of somatotrophic hormone, have been selected for development for high rate of weight gains and these being profitable are preferred in commercial units. Somatotrophic hormone is antagonistic to ACTH which controls the overall adaptation ability of the whole organism and because of close relationship it has unfavourable influence on other hormonal functions (Vigh 1972).

To reach early puberty several experiments have been conducted on prepubertal group of animals. To a group of noncycling gilts when 400 i.u. of PMS and 200 i.u. of HCG was administered simultaneously these expressed ovulatory oestrus and regular cycles (Schilling *et al*, 1971, Schilling & Minar, 1971, Schilling and Cerne, 1972, 73 and Breeuwusma 1974). Fairly good results can be expected in gilts which are due to reach puberty. In such gilts well synchronized oestrus can be induced 3 to 5 days after treatment but intensity of oestrus is poor. In such a lot conception rate is somewhat higher than the controls.

In the domestic sow puberty is reached before the age of one year and some times even as early as that of four months (Corner, 1921). Phillips and Zellar (1943) found breedwise differences and puberty being delayed in smaller breeds. They recorded the average pubertal age in Poland China pigs as 200 days (range 160-250 days).

Warnick *et al* (1950) reported that sexual maturity is delayed by several weeks due to inbreeding. Similar findings have been recorded by Squiers *et al* (1952) and Foote *et al* (1956). Burger (1952) observed that limiting food intake, leading to retarded growth delays sexual maturity. Johnsen *et al*

(1955) recorded delayed puberty due to Vit-B-12 deficiency in pigs.

Age at first oestrus may be influenced by the time of farrowing in relation to the season of the year. Wiggins *et al* (1950) found the average incidence of prepuberal sexual development as lowest in April and highest in October. While their observations in 113 gilts of known age showed that the percentage of immature gilts born early in the Spring was significantly higher.

Plocck (1967) reported in pigs that the optimum age at first mating was 9 to 10 months when the average body weight was 110 to 130 kg. No significant differences were observed in the number of piglings born per litter when the sow was first mated at 8 months or at 15-18 months of age.

### Puberty in Bitches

In bitches, puberty occurs between 6 to 12 months of age. The smaller breeds reach puberty at an earlier age as compared to larger breeds. It is influenced by the environmental conditions as evidenced by the fact that the kennelled animals reach puberty considerably later than the free ones.

### Puberty in Cats

The cats attain puberty between 6 to 15 months. The first fruitful service can occur at about 12 months (Roberts 1956). The first oestrus in cats occurs between 7 to 9 months (Hendrikse, 1965). Algerian cats reach puberty between 15 to 18 months of age (Bourge, 1935; Gross, 1936).

### Factors affecting Puberty

#### Genetic

Age of puberty varies between species, breeds and strains. Cross breeding

minimising the puberty age, whileas indiscriminate inbreeding delays the onset. It is commonly observed that dairy cattle reach puberty earlier compared to beef breeds. Similar observations have been made with regard to draft breeds of cattle under Indian conditions. As compared to the European breeds, Zebu cattle in general require 6 to 12 months more to attain puberty. Influence of sire on the age of puberty of his daughters is recorded in Khillar, Kankrej and Gir cattle (Tatke, 1967, Patel *et al*, 1956 and Velhankar 1973). Ponkshe (1969) reported differences in the age at first heat and first fertile heat between different cow families in Gir cattle but these were not significant.

#### Environmental

**Season:** of birth has an influence on pubertal age and this is particularly marked in sheep and buffaloes which are seasonal breeders. Tatke (1967) observed that Khillar heifers born during March, July and August reach puberty earlier than the herd average of  $33.78 \pm 0.64$  months. The maximum reduction of 3.48 months was observed in heifers born in August while as those born during April reached puberty 4.23 months later. However these differences were nonsignificant.

Sheep are mostly dependant on the season to reach puberty. It is evident that few weeks after the first showers, lambs reach puberty and first signs of oestrus are noticed. Kelly (1946) reported that sexual maturity develops slowly in three stages. During the first stage very few young ewes expressed estrus. During the second stage more ewes exhibited estrus but comparatively very few conceive and during the third stage the majority of ewes come into a

estrus and conceived. It was also shown that delayed development of sexual maturity was usually associated with increased lamb losses and deaths amongst ewes bearing their first lambs. Watson (1953) showed that the time of the year when ewe lambs were born influenced the occurrence of first estrus, the majority of the spring born lambs experience their first estrus between 120 and 200 days of their stage. Winter born lambs experienced their estrus at 201-360 days of their age and summer born lambs at 360-440 days.

### TEMPERATURE

Animals in the temperate zone reach puberty at an earlier age than those in tropical countries. Dall (1959) attributed this to slower rate of growth at higher temperatures.

### NUTRITION

Plane of nutrition has a profound bearing on the onset of puberty. Usually well fed heifers reach puberty at an earlier age than those poorly fed (Joubert 1954, Asdell, 1955, Sorensen *et al.*, 1959, Reid *et al.* 1964, Sundaresan *et al.*, 1965, and Velhankar 1973). Underfeeding has adverse effect on pubertal age. The delay is variable depending upon the type of nutrition. Underfeeding has detrimental effect on the hypophysis which leads to reduced output of gonadotropins.

Overfeeding and obesity also delay the onset of puberty. It is not clearly known as to how overfeeding causes delay in puberty.

Mineral and trace element deficiencies also greatly influence the onset of puberty.

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# Chapter 17

## Oestrus and Oestrous Cycle

In domestic animals which have reached puberty or sexual maturity a new phase of the functional activity of the reproductive system is expressed by the onset of first oestrus followed by met-oestrus and if the animal is not served this transient phase is followed by dioestrus and proestrus. The chain of the physiological events that begin at one oestrous period and end at the next is termed an oestrous cycle.

The rhythm of the reproductive cycle is largely dependant upon the functional activity of the hypophysis and the ovary which leads to several physiological changes in the reproductive tract.

### Hormonal control of oestrous cycle

Hormonal control of oestrus cycle is dependant on the interrelated action of the gonadotropic hormones secreted by the anterior pituitary and the gonadal hormones secreted by the ovary.

Follicular growth and maturation of the ovum is caused by the FSH. As the follicles grow the quantity of oestrogen secreted by them is absorbed in the blood in increasing amounts. Luteinising hormone in conjunction with FSH causes greater stimulus to the theca interna cells of the follicle to secrete oestrogen. The high level of oestrogen suppresses

the level of FSH and the pituitary is stimulated to liberate the luteinising hormone. The luteinising hormone causes the preovulatory enlargement of the follicle, inducing ovulation in spontaneously ovulating animals and subsequent formation of the corpus luteum.

Immediately after ovulation, the secretion of oestrogens is considerably decreased and luteal tissue is developed under the influence of the luteinising hormone in the crater caused by the rupture of the follicle. There is progressive development of the luteal tissue popularly known as corpus luteum which is a temporary endocrine gland. As the corpus luteum develops, there is progressive secretion of progesterone hormone. The level of progesterone reaches its maximum at mid-cycle (10-14 days). Thereafter it declines gradually until the commencement of proestrus period.

The active corpus luteum prevents the growth of any other Graafian follicle during the same cycle. The corpus luteum slowly regresses from about the 18th day onwards thus reducing the level of progesterone which in turn is said to jolt the pituitary to liberate more of FSH, and a new cycle commences with the onset of proestrus when follicular growth occurs under the influence of FSH.

### Neurohumoral control of oestrous cycle

The reproductive phenomenon is dependent on the interaction of neural and hormonal factors. No hormones are released by the pituitary in the absence of neural stimulus since neural impulse is essential for the liberation of hormones. This is evidenced by the fact that the onset of the breeding season in some of the species is related to the day length. Mechanical stimulus as a result of copulatory act leads to the release of luteinising hormone which causes ovulation in some species like the rabbit.

Diseases of the brain and experimental destruction of the hypothalamus is followed by atrophy of the gonads. The possible explanation of such occurrence is that the stimulus of copulation is conveyed by the nervous system to the ventral portion of the hypothalamus. On stimulation, the hypothalamus releases neuro-hormonal substances which are carried by the blood stream to the anterior pituitary and these substances in turn stimulate the release of LH which causes ovulation. Similarly it can be mentioned that the nervous impulse arising from suckling reaches the pituitary in no time in consequences of which there is instantaneous release of oxytocin and letdown of milk. Stimulation of clitoris by massage for a few days may induce oestrus. The hypothalamus is actively involved in control of secretion of hormones by the pituitary.

These changes occur during the various phases which have been divided into proestrus, oestrus, metoestrus and dioestrus period. During proestrus and oestrus periods the ovaries are under the influence of the follicle stimulating hormone for the development of the Graafian follicles and hence this period is called "follicular phase" of the oestrous

cycle. During metoestrus and dioestrus periods, the ovaries are under the influence of leuteinising hormone. This period is known as the "Luteal phase" of the oestrous cycle.

### Follicular phase of the oestrous cycle

#### PROESTRUS

Proestrus is the initial stage of the oestrous cycle. If the animal is not served during the previous oestrus or was served and did not conceive the periodic corpus luteum starts regressing slowly from about the 18th day onwards and more rapidly during the proestrus period (20th & 21st day) as a result of which the level of progesterone drops and the influence of LH is considerably decreased. As a result, during proestrus period the anterior pituitary is activated to liberate more of the FSH. The rise of the follicular stimulating hormone gradually stimulates the development of the Graafian follicle. The ovary secretes oestrogen which is present in the follicular fluid. This is directly absorbed into blood giving rise to gradual changes in the tubular genitalia. There is increased vascularity, and slight rise in vaginal temperature. Externally it can be seen that the vulval lips slowly get turgid and the wrinkles on their surface gradually disappear. Internal changes are observed in the vagina, cervix and the uterus with marked vascularity. Congestion of the vestibular portion of the vagina is pronounced. During this period if the ovaries are palpable one or more Graafian follicles can be palpated on either of the ovaries; which continue to increase in size as the period progresses. A certain amount of excitement is also noticed. Pro-estrus phase is also known as "building-up



period" because of increase in (a) vascularity, (b) muscular activity, (c) mucus secretion and (d) growth of epithelial tissue of the reproductive system that occurs during this phase.

The duration of pro-estrus period varies from species to species, the range being 2 to 3 days.

## Oestrus

When the oestrogen content of blood reaches an optimum level, further peculiar changes occur giving rise to external visible symptoms and marked internal changes. The proestrus period is now merged with the oestrus period. The high level of oestrogen leads to psychic manifestations. The animal becomes receptive to male and is said to be in heat. This period is characterised by the manifestation of sexual desire. The duration of this stage varies from species to species.

During oestrus, the genitalia is under the tropic action of oestrogen giving rise to development of its various parts such as vagina, cervix, uterus, fallopian tubes and also the mammary tissue in heifers. The tone of uterus is increased and gradual relaxation of cervix occurs. Further progressive changes occur particularly with the manifestation by the voidance of oestral discharge. The discharge also acts as a lubricant to vaginal passage during copulation.

## Luteal phase

### MET-OESTRUS

Oestrus is followed by met-oestrus period which is characterised by the cessation of heat symptoms. Duration of met-oestrus is variable. The excitement in the female gradually disappears and the termination is marked by refusal to accept the male. There is a perceptible

decrease in the quantity of discharge voided and a change in the consistency is also noticed. The uterus is tonic with relaxed cervix.

When the oestrogen level has reached its maximum, production of FSH by the pituitary is suppressed, in consequence of which LH is increased in quantity. The LH in conjunction with FSH and oestrogen causes ovulation. Corpus luteum is formed thereafter which starts secreting progesterone.

During met-oestrus there is hyperleucocytosis and the vaginal epithelium shows morphological changes. Post-oestral bleeding may occur during the early part of met-oestrus when epithelium of the uterine caruncles becomes very hyperaemic and capillary haemorrhage may take place both by diapedesis and capillary wall rupture. The endometrial glands are progressively activated with endometrial proliferation and thus the uterine bed is prepared for reception of the fertilised ovum.

If pregnancy does not supervene, then the reproductive tract goes into a resting stage called dioestrus.

## Dioestrus

In domestic animals this is the longest stage of the oestrous cycle. Luteal activity is at its maximum during this phase. There is thickening of the endometrium and hypertrophy of the uterine glands. The cervix is constricted and the vaginal secretions decrease and become more sticky. The vaginal mucus membrane changes from pink to pale. During the latter half there is relaxation of the uterine musculature. This is the quiescent stage of the oestrous cycle otherwise known as the resting stage. The high level of progesterone and its prolonged effects sup-

press the level of FSH and does not allow ovarian follicles to develop. During the declining phase of the dioestrus, corpus luteum slowly begins to regress and the progesterone activity is reduced, giving rise to a slow development of the primary and secondary follicles and the regression of the corpus luteum approaching proestrus.

#### PATTERNS OF OESTROUS CYCLE

According to the number of oestrous cycles, the animals are divided into three groups — monoestrous, polyoestrous and seasonally polyoestrous.

##### Monoestrous

In this group females exhibit one oestrous cycle in a year. Wild animals fall in this category. Bitches also manifest monoestrous cycles. Most of the breeds exhibit one to two breeding seasons in a year.

##### Polyoestrous

In this group, females exhibit regular cycles throughout the year. Cows and goats fall in this category.

##### Seasonally Polyoestrous

In this group females exhibit oestrous cycles during certain seasons only and in other seasons they manifest long anoestrus. Mares and ewes of certain breeds experience such seasonally polyoestrus conditions. Majority of the buffaloes in Northern India also show similar trends. They are usually anoestrus from April to July or August.

#### OESTROUS CYCLE IN THE COW

The cow is polyoestrous and regular oestrous cycles are exhibited throughout the year. The length of one oestrous

cycle on an average is 21 days. A considerable variation is observed in the duration of pro-oestrous, oestrous, and metoestrus periods. Proestrus may vary in length from 48-72 hours. The duration of oestrous may vary from 6-24 hours. Under the continental conditions, the length of oestrus is greater in Spring and early part of Summer. Under Indian conditions, oestrous cycles in cows are of a longer duration from October to April and of shorter duration during Summer months. Metoestrus may last for about 48 hours.

The detection of heat in cows is very important since breeding under controlled conditions is largely dependent on the service in good time by natural mating or by Artificial Insemination under various programmes for Cattle Development.

Great variations are noticed with regard to the signs of heat, since the exhibition of oestrus is dependent upon variety of factors such as hereditary, environmental, managemental and nutritional. The duration of oestrus observed in Indian and exotic breeds of cattle is presented in Table 22.

#### INTENSITY OF OESTRUS

The intensity of oestrus varies from cow to cow as weak, medium and intense. In the majority of cows, heat is detected during morning hours. The percentage of cows that come on heat during afternoon or evening hours is comparatively small.

Choudhary *et al* (1965) observed in Haryana heifers that only 3.33 per cent were intense, 42.52 per cent were medium and 54.15 per cent weak in intensity of oestrus. Hukeri (1965) observed the intensity of oestrus as pronounced, medium and weak in 23.22%, 65.4% and 11.37% respectively.

Table 22  
DURATION OF OESTRUS IN THE COW

Breed	Duration of Oestrus in hours		Reference
	Average	Range	
Sahiwal	—	4 to 15	Kumaran (1951)
Sahiwal	—	8 to 24	Kaikini (1975)
Tharparkar	14.36 $\pm$ 0.44	—	Hukeri (1965)
Tharparkar	—	6 to 21	Kumaran (1951)
Kankrej	12.58	—	Patel (1953)
Hariana	23.09 $\pm$ 0.92	—	Ahuja <i>et al</i> (1961)
Gir	—	12 to 16	Kodagali (1975)
Red Sindhi	—	18 to 24	Sane (1975)
Jersey	11.54	—	Ulloa (1954)
Holstein-Friesian	10.09	—	Hall <i>et al</i> (1959)

### Onset of Oestrus and Ovulation

Choudhury *et al* (1965) reported the onset of oestrus in Hariana cows as 42.53% during morning, 21.31% during afternoon and 36.08% during night hours. Hukeri (1965) observed the onset of oestrus in Tharparkar cows as 60.68%, 25.59% and 13.74% during morning, noon and night hours respectively. Sonawane (1969) found equal proportion of Rathi and Gir cows coming on heat during 6 a.m. to 12 noon and between 12 noon to 6 p.m. but none during the night hours. Ponkshe (1969) observed majority of Gir cows studied by him coming in heat between 4 a.m. to 12 noon. Madan and Razdan (1966) reported the onset of oestrus in Red-Sindhi, Sahiwal and Tharparkar cows, to be least during the night hours and maximum during morning hours with a decrease in the afternoon and evening hours. The largest proportion came on heat between 6 to 10 a.m. The differences were signi-

ficant. They further found that the ovulation time was shorter when the onset of oestrus began in later hours of the day. Madan and Razdan (1966) have also reported in Red-Sindhi, Sahiwal and Tharparkar cows that the average intervals between onset of heat and ovulation were 26.56, 26.80 and 27.12 hours respectively. The modal interval for ovulation for all breeds was 27 hours. A total of 57.03% ovulations occurred between 25th and 28th hours. The mean time taken for ovulation was 26.91 hours. Their observations on 94 Red Sindhi, Sahiwal and Tharparkar cows showed that when oestrus commenced between 4.00 to 10.00 hours, the mean ovulation time was 27.01 hours; the lowest average of 25.91 hours was recorded for cows coming on heat between 16.00 to 22.00 hours. The animals in which oestrus occurred during night took longer time to ovulate (28.00 hours). More number of heats were detected during morning hours.

### External signs of oestrus

Cows in intense heat may exhibit partial inappetance due to excitement. The animal is not keen on feed and while in stables prefers standing. There is a drop in the milk yield particularly in those exhibiting intense heat symptoms. Restlessness is observed in variable degrees. If one enters the byre quietly a cow in heat is generally found standing with frequent attempts at micturition with raised tail and crouching the back and lumbar region. Bellowing is marked. The vulval lips appear swollen and congested, the wrinkles on the vulva disappear and the vulval lips appear turgid and stand prominently. Depending on the stage of oestrus, a clear, shiny mucous discharge is seen at the vulval lips extending down to the perineal region and smearing the hindquarters. (Fig. 65 a, b). The film is thin and transparent. Discharge may be seen hanging from the vulval opening right down to the feet. It is stringy, having good consistency and is odourless.

When a cow in oestrus is either let out in the open or on pasture along with other cows, it is usually observed that she is nervous, moves anxiously in the field and bellowing becomes marked with an anxious expression. Homosexual behaviour is also markedly observed. The cow on heat is mounted by other cows and in turn she attempts to ride other cows. In certain cows mounting and copulatory act except thrust is exactly the same as would occur during the service of a bull. The vulval lips of the cow in heat are sniffed by other cows. There is a slight rise in the body temperature according to the intensity of the oestrus.

The cow in good heat is not interested in grazing. The symptoms are well marked in the proximity of a bull. Due

to the excitement, the cows in heat like to free themselves from the stanchion and rings.

### Oestrous Discharge

The oestral discharge varies in consistency and quality during different stages of oestrus. In the beginning it is thin, shiny, clear and seen hanging out of the vulval opening.

During proestrus, there is an increase in the number of mucus secreting cells of the cervix and later of the vagina. The discharge is at its maximum during oestrus when abundance of mucus is usually noticed in the fornix vaginae. During oestrus the mucus is copious, stringy, adhesive and transparent, occasionally containing exfoliated cells.

### Changes in Vaginal Epithelium

During oestrous cycle constant changes are occurring in the vagina due to the influence of the ovarian hormones — oestrogen and progesterone. Histological changes are entirely dependent on the hormonal levels. The growth and thickening of the vaginal epithelium during oestrus is under the influence of oestrogen. It changes to thin, low, cuboidal type in the luteal phase of the cycle after ovulation. During dioestrus, the superficial epithelium varies from flattened to low columnar type.

### Changes in the cervix

The surface epithelium becomes uniform in height and mucus is secreted by few cells, a week after oestrous. At about 18 days, the epithelial cells become larger and there is commencement of mucus accumulation. During the next oestrus, the epithelial cells assume larger size than at any other stage of oestrous cycle and their surface is covered by a thin mucus layer.

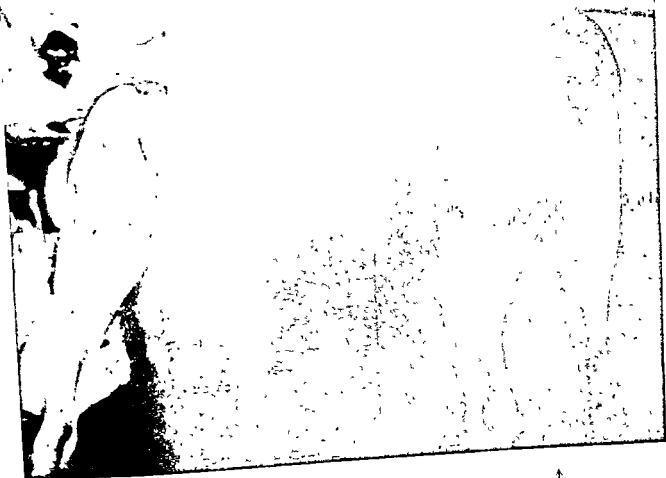


Fig. 65 a. Vulval oedema during oestrus. Note the mucus discharge.



Fig. 65 b. Vulval oedema during oestrus. Note the mucus discharge.



Fig. 67 a. Placentation in buffalo — cotyledonary.



← Fig. 67 b. Placentation in buffalo — cotyledonary.



← Fig. 67 c. Placentation in buffalo — cotyledonary.



Fig. 68. Maternal caruncles in a pregnant buffalo.



Fig. 70. Maceration of foetus in right cornua in a buffalo.



Fig. 71. Maceration of foetus in left cornua.

## Length of Oestrous Cycle

Anderson (1914) recorded a mean length of  $23.03 \pm 0.19$  days in Zebu-cattle, the modal length being 22 days. Luktuke *et al* (1953) reported the mean length of oestrous cycle in Sahiwal heifers as  $21.00 \pm 0.07$  days and  $21.5 \pm 0.08$  days in Sahiwal cows. Patel (1953) reported the range of oestrous cycle as 15.57 to 22.9 days in Kankrej cows; the cycles being shortest in Spring and longest in Winter. Differences between Summer and Autumn were negligible. Ahuja *et al* (1961) recorded oestrous cycles in Hariana heifers as  $22.6 \pm 0.36$  days and  $20.32 \pm 0.31$  days in Hariana cows and no seasonal effect was seen. Arora and Gupta (1963) reported that the mean length of oestrous cycle in Nimar cows was  $21.90 \pm 11.94$  days. Bhattacharya *et al* (1964) reported their observations on heifers of certain Indian breeds. (Table 23).

Differences between breeds and within heifers of the same breed were not significant. Chaudhari *et al* (1965) reported the mean length of oestrous cycle in Hariana heifers as  $20.55 \pm 0.27$  days. Purbey (1965) reported the mean length in Dangi cows as  $20.4 \pm 0.19$  days. Tatke (1967) recorded the mean length of oestrous cycle in Khillar cows as

$22.26 \pm 0.03$  days. The cycles were of short duration in Summer (19.95 days) and longest in rainy season (20.57 days). Hukeri (1965) reported in Tharparkar cattle the mean length of oestrus cycle as  $20.65 \pm 0.2$  days. Sane *et al* (1968) reported in Gir and Red Sindhi cattle the mean length of oestrous cycle as  $19.10 \pm 0.51$  and  $21 \pm 0.33$  days respectively. Ponkshe (1969) reported in Gir cattle the mean length of oestrous cycle as  $20.85 \pm 0.30$  days. He further observed that the differences between different cow families were highly significant.

Quinlan *et al* (1941) reported average duration of oestrous cycles in Friesian and Red Poll breeds as 19.8 days. Chapman and Casida (1937) found that 60% of clinically normal cows had oestrous cycle of 17 to 24 days. Asdell (1946) reported a modal length of oestrous cycle of 22 days for heifers and 21 days for dairy cows. Musgrave (1957) recorded overall oestrus cycle length of 41.66 days in Holstein, Guernsey, Ayrshire and Jersey cows of which 41% cycles fell in the range of 18 to 24 days. Erb *et al* (1958) recorded the modal length of oestrous cycle as 21 days with 72% falling within the range of 18 to 25 days in Holstein cows. Rollinson (1963) found the mean oestrous cycle length in Nganda and Zebu cattle as  $20.9 \pm 1.38$

Table 23  
LENGTH OF OESTROUS CYCLE IN INDIAN CATTLE

Sr. No.	Breed	No. of Animals	No. of oestrous cycles	Length of oestrous cycle days	Range days
1.	Sahiwal	8	120	21.09	17 to 26
2.	Gir	8	119	21.01	16 to 32
3.	Hariana	8	119	21.18	17 to 29
4.	Tharparkar	7	100	21.39	16 to 32
5.	Red-Sindhi	7	109	21.21	16 to 35

days with a range of 18 to 24 days in 61.7% of the cycles

### Ovular and Anovular Oestruses

Choudhary *et al* (1965) observed in Haryana heifers a high incidence of 56.58% anovular heats. Hukeri (1965) reported in Tharparkar cattle that 29.38 per cent of heats were anovular where as 33.34 per cent of first postpartum oestruses were anovular. In Dangi Gir and Rathu breeds an incidence of 15.8 per cent anovular heats was recorded by Purbey (1965) and Sonawane (1969).

Rakha *et al* (1970) observed the percentage of anovular heats as 39, 69, 47 and 20 in Angoni, Mushona, Africander and Hereford heifers.

### Post oestral bleeding in the cow

Post oestral bleeding is encountered in the cow, dog and guineapigs (Asdell, 1946). The post oestrus bleeding in the cow is a process of diapedesis with some denudation of the endometrial epithelium particularly in inter caruncular spaces (Weber *et al*, 1948). In the cow the appearance of blood in the oestral discharge is rare and the flow is clear and transparent. The post oestrus bleeding in the cow occurs immediately after ovulation i.e. about 12 to 24 hours after the cessation of oestrus.

Post oestrus bleeding in cows occurs at 1 to 3 days post oestrus in about 90% of the cycles in young heifers and in about 50% of the cycles in the cows (Hansel and Asdell, 1952; Lagerlof, 1965).

Post oestrus bleeding may be observed irrespective of conception occurring or otherwise and it does not interfere with conception (Trimberger, 1941). The lower incidence of post oestrus bleeding may be due to the endometrial

arterioles being highly coiled in multiparous cows (Hansel and Asdell, 1952). Microscopic bleeding in the normal cow may be due to progressive rise in levels of progesterone and its occurrence at about 10 days post oestrus is probably due to the action of latent period (Cole 1930). Weber *et al* (1918) reported incidence of post oestral bleeding in virgin heifers of continental breeds as 81% varying from scanty red coloured mucus to copious bleeding which was noticed 50 to 60 hours after the onset of oestrus. Post oestrus examination of heifers in which there was no evidence of microscopic bleeding revealed either petichial, ecclumatic or diffuse haemorrhage confined to the caruncles and endometrial oedema reaching its maximum at the ovulation time. The uterine epithelium undergoes changes in colour during proestrus to oestrus from yellow to brown pink. Das (1965) reported post oestrus bleeding in zebu cattle from Malaysia in about 20 to 25% of cases.

Sane and Kaikini (1953) and Kaikini (1969) reported 2% post oestrous bleeding in Red Sindhi and Sahiwal. Antrup and Rasbech (1951) and Larson and Bayley (1955) reported that 20 to 30% conceive when bred at metoestrus bleed. Ovulation is not a prerequisite to post oestrus bleeding since bleeding sometimes starts before ovulation. During coitus the vagina is likely to be injured and capillary bleeding may result which should not be mistaken for post oestral bleeding. Similar injuries are likely to occur by faulty use of pipette during inseminations and also particularly when speculum is used to dilate the vagina.

### GESTATIONAL OESTRUS AND OVULATION DURING PREGNANCY IN COWS

There is cessation of oestrous cycles following conception.



The occurrence of oestrus during pregnancy in cows though rare, is not uncommon. Gestational oestrus is of practical importance because, in the absence of accurate knowledge of pregnancy diagnosis the cow may be disposed off as a repeat breeder or there are chances of induced abortion due to service.

Certain number of cows exhibit estrus during the first three months of gestation. It may even occur upto 6 months of pregnancy. Pregnant cows exhibiting oestrus may accept service. Abortion is likely to occur if pregnant female in oestrus is served naturally or by artificial insemination. It is therefore necessary to ensure that pregnant females should not be left loose with the bull.

During early period of gestation, usually there is considerable follicular development in the ovaries. Few follicles may reach the ovulatory size. Such follicles remain longer and then become atretic. During the late period of gestation, follicular development declines both in number and size. This decline is possibly due to pituitary gonadotrophin suppression as a result of increasing amounts of estrogen and progesterone secreted by the placenta.

Bullard (1934) reported gestational oestrus in heifers and cows upto fifth gestation period. It occurred one or more times between 65 to 235 days at first gestation and 31 to 253 days in subsequent gestations. The maximum recurrence being twice during the first and four times in the subsequent gestations. Donald (1953) reported that gestational oestrus was observed in 3 to 4% cows. This was irrespective of breed and age. Sex of the calf had no influence. Erb and Morrison (1958) reported the incidence of gestational oestrus in 5.6% of gestational periods in Holstein-

Friesian cows. The average interval of gestational oestrus was  $43 \pm 1.9$  days. Hall *et al* (1958) observed the incidence of post pregnancy oestrus in 2.22% dairy cattle. His further observations on Louisiana cattle indicate gestational oestrus to the extent of 14.4% in cows and heifers and 3.4% of the oestrus periods studied. Rahmann and Mead (1958) reported the incidence of post conceptional oestrus which varied from 2.07% to 7.75% in Jersey cows. It occurred 162 times in 147 gestational periods of 141 cows. The average length of post conceptional oestrous cycle was 63.0 (11 to 213) days. Hafez (1962-1968) reported that about 10% pregnant cows may show gestational oestrus and this is of great practical significance. It mainly occurs in 3 to 5% pregnancies during the first three months (Donoho and Rickard, 1955; Hafez, 1968). Luktuke *et al* (1964) reported the incidence of gestational oestrus in Hariana cattle as 7.61% in cows at an average of  $113.2 \pm 6.5$  (7 to 278) days of pregnancy. Oestrus occurred in all stages of pregnancy but mostly within 180 days of gestation. The intensity of gestational oestrus was pronounced in 1.9%, normal in 72.1% and weak in 26.0%. Choudhary *et al* (1965) reported the incidence of gestational oestrus in 13 (10.65%) Hariana heifers, at an average of 131.44 (69 to 180) days for first gestational oestrus and 149.75 days for second gestational oestrus which occurred 23.25 days following the first gestational oestrus. Of the gestational oestrus exhibited, 77% were weak in intensity. Hukeri (1965) reported in Tharparkar cows the incidence of gestational oestrus as 8.84% in cows and 5.68% in 201 oestrus studied. It occurred at an average interval of  $143.91 \pm 19.39$  (57 to 212) days from

conception It was observed twice in 16.67% or 12 gestational oestruses All the gestational oestruses were anovular Sane *et al* (1968) recorded only 2 cases of gestational oestrus in 142 Sindhi cows (1.4%) This was observed to occur within first three months of gestation Kaikini *et al* (1974) recorded an incidence of 4% gestational oestrus in Sahiwal cows Kaikini and Pargaonkar (1975) recorded incidence of 3% gestational oestrus in non-descript cows

### BREEDING SEASON IN BUFFALOES

In buffaloes, the breeding season is more or less restricted to certain months of the year although in few, heats are observed in other months Dave (1938) reported in Surti buffaloes that sexual activity was observed from August to November Ocampo (1939) reported that in Philippines maximum sexual activity was observed between August to January Ishaque (1957) reported that buffaloes are seasonally polyoestrus expression of heat being limited to eight months of the year and sexually in active condition is observed from March to June, when hardly 3% of the heats are recorded The peak season for oestrus in buffaloes is reported to be from October to February Goswami and Nair (1964) reported that in Indian buffaloes more conceptions occur when both diurnal temperature and relative humidity are low i.e. during winter High environmental temperature affects the conception rates adversely Breeding efficiency in male buffaloes is reported to be at the peak period during winter with similar observations in buffalo-cows It is observed that calving season in buffaloes is not conducive for conceptions probably on account of the reduced

activity during the period when high air temperature in association with high relative humidity is prevailing Wang (1965) reported in Nanking buffalo cows, that highest number of heats recorded were from September to December, the seasonal trend being significant El Shaikh (1967) reported that in Egyptian buffaloes, the breeding season extends throughout the year with no anoestrus periods However 60.79% of the calvings occurred between August to January Based on a total of 708,131 inseminations in Surti buffaloes during a period of 5 years (1967-72) Kodagali *et al* (1973) reported that 69.62% of the buffaloes were bred during the months of September to February when the average minimum temperature was 61.29°C (50.8 to 94.3°F) Further 30.38% of the buffaloes were bred during the months of March to August when the average minimum and maximum temperatures were 77.27°C and 94.39°F respectively (range 66 to 81.23°F and 86.78°C to 103.49°C) Highest percentage (14.45) of buffaloes were bred during the month of December whereas lowest percentage (2.71) were bred during the month of May Venkatraman and Venkayya (1911) reported that 81% of calvings in Murrah buffaloes occurred during the monsoon seasons Dominique (1951) reported from Brazil that in Amazon, calvings in buffaloes mostly occurred from September to December with the maximum services recorded from January to March Calving season in Central Brazil is mainly from January to April Ragab and Asker (1951) reported in Egyptian buffaloes that the maximum calvings occurred during August to March Arunachalam *et al* (1952) reported that calvings in Murrah buffaloes were maximum in October and November and

least from February to April. Alim and Ahmed (1954) reported in Egyptian buffaloes that the major number of calvings occurred from September to March. Asker and El Itribi (1958) reported that 47% of Egyptian buffaloes calved between October to January. Singh and Sharma (1958) found that in Murrah buffaloes calvings occurred from June to December. In India and Pakistan water buffaloes show marked seasonal variation in calvings, with a peak from August to October, the maximum being in September (Rife, 1959). Kohli and Mullick (1960) reported in Murrah buffaloes that calvings occurred throughout the year but the maximum recorded were from July to November and least in January and February. Nambiar and Raja (1962) reported in Murrah buffalo-cows in Kerala that maximum number of calvings were recorded from August to January (67.2%) — highest being in September (15.05%) and lowest being in April (3.23%).

### Oestrous cycle in Buffaloes

Rao and Murari (1956) reported in Murrah buffaloes that the length of oestrous cycle was 22.9 days. Ocampo (1939) reported that the oestrus symptoms in buffaloes are more intense than those in cattle. The duration of oestrus lasts for about 1 to 1½ days. The length of oestrous cycle is about 21 days and is more variable than in the cow. El Shaikh (1967) reported that the average length of oestrous cycle in Egyptian buffaloes was 27.65 days. The modal length of oestrous cycle ranged between 16 and 21 days. The duration of oestrus is comparatively longer in the buffalo than in cow. Usually it lasts for about 24 hours or more (Kaleff, 1942; Bhattacharya, 1953; Hafez, 1954). Wang

(1965) reported in Nanking buffaloes the various stages of oestrous cycle, pro-oestrus 15 hours 43 minutes, oestrus 22 hours 6 minutes, and metoestrus 15 hours 42 minutes. He further reported that the ovulation takes place at about 4½ hours after the end of heat. Basirov (1964) recorded that in buffaloes in Azarbaijan, oestrus lasts 20 to 35 hours and ovulation occurring 33 to 40 hours after the onset of heat. Shalash (1958) reported that in Egyptian buffaloes, the average duration of oestrous cycle was  $21.6 \pm 0.23$  days. There was highly significant variation between months and seasons in the length of the cycle. The average duration of oestrus was 11.92 hours and ovulation time ranged between 18 to 44 hours from the onset of heat. Dominiques (1961) reported in Brazilian buffaloes the length of oestrous cycle from 18 to 28 days. The duration of oestrus was from 6 to 48 hours. Hafez (1954) found that in Egyptian buffalo the onset of oestrus was between 6 p.m. and 6 a.m. in 84% cases studied. Mac Gregor (1941) observed that in the Swamp buffaloes oestrus symptoms are not intense during the day and mating usually takes place during night hours. Silent heat is commonly observed in the buffalo (Marathe and Sane, 1964).

Most of the workers in India reported the average length of the oestrous cycle as 21 days.

Villegas (1959) reported that in the Philippines, high sexual activity is observed in buffaloes during the rainy season and cooler months. In Egypt, Hafez (1955) and Asker and El Itribi (1958) observed that majority of the buffaloes remain in anoestrus condition during summer months (April to July) and they return to sexual activity in Autumn (August), and majority of services occur from November to February.

It has been observed by various workers under Indian conditions that about 80% of the total number of oestrous periods recorded in buffaloes were from October to March and only 20% during the period from April to September. The higher temperature and humidity coincide with the period of lower sexual activity (Roy *et al*, 1962, Goswami and Nair, 1964).

Pargaonkar and Karkani (1968) and Pargaonkar (1969) recorded the length of oestrous cycle and duration of heat period in Nagpuri (Berari) buffaloes as 33.7 days and  $28.61 \pm 0.51$  hours, respectively.

### BREEDING SEASON IN MARES

In Britain and Canada, the seasonal cycles begin by about March and continue until the end of August, the peak period being in May. In Australia and New Zealand there is peak season in October and November. Osborne (1966) stated that the breeding season in Australia extends from September to December. This is suggestive of the fact that there is a correlation between day light and sexual season. The oestrous cycle usually starts during the early part of the spring season and in unbred mares the cycles continue well into the Summer. Hammond (1936) reported that major number of foalings occur during the spring season. With the influence of domestication breeding is also possible during autumn and winter. Ganowicz (1965) reported that breeding season extends between February to May under Russian conditions. Hadi (1966) reported that the mares bred from April to October exhibited heat throughout the year, but fecundity was low during the first three and last two months of the year.

### OESTROUS CYCLE IN THE MARE

The mare is seasonally polyoestrus. The occurrence of oestrous cycle in the mare is dependant upon the season and environmental conditions. The average duration of oestrus in the mare is about 5 to 7 days. It is observed that oestrous periods are of a longer duration in the beginning of the breeding season. The duration of oestrous cycle varies from 18 to 21 days. If the animal has not conceived, the second period on an average will occur 16 days from the time of cessation of heat. In the mare, proestrus usually lasts for 3 days, oestrous 5 to 7 days and the metoestrous and dioestrus periods are variable. Hadi (1966) reported that no regular rhythmic cycles occurred in the Deccan mares. Considerable irregularity in oestrous cycle was a constant feature in these mares.

### Diagnosis of oestrus in the mare

In the mare, it is usually observed that there is a change in the temperament during the oestrus period which is particularly noticed in working mares. A number of mares do show vicious temperament. The exhibition of heat symptoms is not so well marked as in the cow. There is frequent micturition and a brownish yellow discharge from the vagina. This is particularly conspicuous in the presence of a stallion or a teaser. When there is vulval relaxation erected clitoris is prominently observed which is known as 'Winking of the clitoris'. A stallion is daily shown to the mare to detect whether she is in heat or otherwise. The vulval mucus membrane is congested becoming orange or scarlet in colour. It looks glossy and is covered with a transparent mucus film. The mucus membrane of the vagina becomes highly vascular and watery mucus may accumulate in the

vaginal fornix. The cervix is considerably relaxed and two to three fingers can easily be inserted. During oestrus, the mare will turn her back to the stallion and stand quietly with tail raised on one side. This is usually a sign that the mare is in heat. The mare is receptive to stallion from 3 to 7 days depending on the duration of estrus.

Asdell (1946) in his extensive review reported an average cycle length of 22 days varying from 19 to 23 days. Hammond (1938) has reported that in immature and senile mares, oestrus may last from 10 to 15 days with metoestrus and dioestrus period lasting for about 16 days. Day (1939) found that under conditions in Great Britain when food supply is limited during Spring and Autumn, mares usually experience long oestrus and dioestrus periods. Blood is occasionally seen in the proestrous discharge of mares.

### Foal heats

It is usually considered that foal heat is the appropriate time to breed a mare. Trum (1950) observed that though there is wide variation between foaling and first heat, 77% of the mares were observed on heat between seventh to tenth day. Hadi (1966) reported the average period of foal heat as 10.1 days. Arora (1970) recorded the average interval of foal heat as  $6.95 \pm 0.13$  days in mares and  $8.58 \pm 0.86$  days in donkey mares, the corresponding C.R. being 33.77 and 42.22 per cent respectively.

### BREEDING SEASON IN EWES

Grant (1934) reported that the cycle in scotch black faced ewes lasts for about 16 days and oestrus for two days. The Suffolk X Leicester — Cheviot sheep in Britain have breeding season from

October to late March. (Hammond, 1944). Marshall (1942) pointed out that sheep transferred from one hemisphere to the other adjust themselves rapidly to the change of season.

Nambiar (1958) reviewed that the following breeds of sheep in India, breed all the year round — Bellary, Bikaneri Hassardale, Deccani, Finewooled and Mandya. Dunn (1960) during his comparative studies has shown that autumn is more favourable breeding season than Spring in New South Wales (Australia), the conception rate for Autumn mated ewes being 10% higher than spring mated ewes. Lambing percentage strongly favoured autumn mating as observed from 15% more lambings, 25% more twinings and 37% more lambs mothered. The weaning weight of autumn born lambs tended to be slightly more than spring born ones. Singh and Sharma (1952) considered the best season of mating sheep under Indian conditions is from March to May and lambing from August to October. Asdell (1946) reported the mean cycle length as 16.5 days. In Merinos and Rambouillets, the average length was about a day longer. McKenzie and Terrill (1937) found that the duration of oestrus in sheep was normally from 3 to 73 hours, with a mean of 21-27 hours. Cole and Miller (1935) reported that in American Rambouillet, the duration of oestrus was two days. Under Indian conditions, sheep have three breeding seasons — 50 to 80% in June, July and August, 15 to 20% in March and April and only a small percentage in October and November (Khot, 1957). Summer matings produce best lambs as more nutrition is available at weaning age. Summer lambs are usually a loss. Singhvi (1964) reported that in Jaisalmeri Sheep, there are two breeding

seasons — one from March April extending to a part of May and the other of August September

Roy *et al* (1962) reported that the natural breeding season in Bikaneri sheep is during July to August. They reported the influence of managerial conditions on the breeding season. Removal of all rams from the flock at the end of August and their reintroduction into the breeding flock immediately after the first few showers of monsoon has beneficial effects. During three or four weeks preceding the monsoon, the concentrate feeding to the female stock is to be slightly raised and the flock permitted to graze or browse for nearly a fortnight on freshly sprouted leaves and grasses. Vasectomised teaser has to be reintroduced in the flock and within a period of 30 days, over 90% of sheep usually come on heat. Roy *et al* (1962) also succeeded in synchronising 60% of the heats in ewes over six days by the middle of the 30 day period. Segregation of ewes from males for a period of nine months from September to May if maintained on a slightly elevated plane of nutrition during June and if given access to the new grass sprouting after the first few showers will enable the ewes to exhibit heat within three weeks time.

Ram Murthy (1963) noticed that Nilgiri ewes came on heat regularly throughout the year and hence it was found better to have two mating seasons — one in the months of March April and another in August September. Sahani and Roy (1967) reported the non character of sexual activity in Bikaneri sheep. Bhasin and Desai (1967) recorded that nearly 80% of Bikaneri sheep at Hissar come into breeding in the month of March. Tyagi and Lavania (1969) reported that 59.8% indigenous

sheep at Pashu Lok Farm (U.P.) were bred from June to September and 39.9% from October to January. They found that more indigenous ewes are bred during higher diurnal temperature whereas the cross bred (Polwarth 1/2 to 3/4) ewes breed more when the atmospheric temperature was low. Sahani and Roy (1972) studied the performance of Bikaneri ewes at the CSWRI, Rajasthan (India) and reported that the fertility was optimum and season had no influence on lambing rate of Bikaneri, Mandya and 1/2 bred ewes when mated with rams of similar breed. Taparia (1972) reported all year round breeding in Sonadi ewes. However, the frequency of breeding tended to be low during November, December and June. The ewes were observed in heat from October to April and mated in November. Tiwari *et al* (1973) reported that Rambouillet and their crosses with Malpura and Chokla behaved exactly in the same manner as the native ewes during autumn season. They concluded that Rambouillet and their crosses with native sheep would show optimum sexual activity during cooler part of the year under semi arid conditions.

### Oestrous cycle in ewes

Sheep are generally polyoestrus in tropics however effective breeding is only possible during restricted seasons of the year. Lambs are born during the most favourable season when food is available in plenty. Jha and Biswas (1964) consider rainy season to be the best for lambing due to availability of ample greens. In Great Britain most of the breeds are seasonally polyoestrus except the Dorsethorn which cycles all throughout the year. Under continental conditions, oestrus cycles commence in autumn and continue upto January.

Merino, Karakul and Dorset horn breeds have oestrous cycles mostly throughout the year. On an average, duration of oestrous cycle in the ewe ranges from 15 to 19 days. Prooestrous period in ewes is not well defined and is usually short, since growth of Graafian follicles is very rapid. The average length of oestrus period under continental conditions is 36 hours. Variations in the duration of oestrus are observed from tract to tract. A large proportion of Australian Merino ewes have short oestrus periods, the duration of which is less than 19 hours. In Bikaneri sheep, the duration of oestrous cycle is  $17.1 \pm 0.12$  days (Roy *et al*, 1962). Sahani & Roy (1967) confirmed similar observations in Bikaneri breed. They recorded oestrous cycle ranging from 14 to 18 days with an average of 17 days. The incidence of short (14 to 15 days) and long (19 to 20 days) oestrous cycles was low (4 to 5). In the Sonadi breed of sheep Taparia (1972) recorded oestrous cycle length ranging from 15 to 19 days with an average of 17.2 days. It ranged between 16 to 17.5 days in 85.6 per cent cases studied.

Reproductive behaviour of Jaisalmeri, Sonadi, Pugal, Magra and Nali breeds of sheep maintained on high and low plane of nutrition was studied and observations on oestrous cycle length recorded. The duration was  $17.78 \pm 0.122$  days in concentrate fed and  $17.57 \pm 0.126$  days in non-concentrate fed groups. No significant differences were observed between breeds and between treatments (Honmode, 1971).

Tiwari *et al* (1973) studied oestrous cycles in Malpura, Chokla and Rambouillet cross sheep. The cycles ranged between 17 to 19 days in these breeds the maximum number of cases falling

between 17-18 days. The incidence of short (13 days) and long (21.42 days) cycles varied in different breeds from 0.3 to 9.8%. However, in Rambouillet and cross breeds there was a marked tendency for the cycle lengths to occur between 16th and 17th day (Table 24).

### Detection of oestrus in ewes

Restlessness is marked to a certain extent. There is progressive swelling of the vulval lips. The symptoms become conspicuous in the presence of a ram. The ewe in heat will stand to the ram and accept mating. While at pasture, the ewe in heat may show frequent mic-turition and may isolate herself from the rest of the flock (Hulet, *et al*, 1962). It is difficult to detect ewes in heat and as such difficulty always arises to spot them particularly when breeding by artificial insemination is planned. The usual practice followed to spot out the ewes that are served is by applying colour crayons on the brisket of the ram. Ewes that are served by the ram get the colour on their hind quarters. During oestrus, irregular cornification of the surface cells of the vagina is noticed. There is moistening of the vagina with the progressive mucus secretion. However, the oestral discharge is not abundant as in the cow. Uterus is congested with enlargement of the uterine glands. Black patches of melanin pigment are commonly seen on the endometrium.

Sahani and Roy (1967) recorded the duration of oestrous in Bikaneri ewes to be 12 to 24 hours. The number of ewes manifesting silent heat was more in the winter months indicative of slight quiescence in sexual activity during this season. In view of the overall low incidence of silent heats recorded in these studies, a 42 to 45 day non-

Table 24  
VARIATIONS IN THE LENGTH OF OESTROUS CYCLES IN  
DIFFERENT BREEDS OF SHEEP

Sr No	Breed	Range (days)	Average (days)	Reference
1	Bikaneri	16 18	17 1	Sahani & Roy (1967)
2	Sonadi	15 19	17 2	Taparia (1972)
3	Jaisalmeri	13 20	17 9	Tiwari <i>et al</i> (1973)
4	Malpura	13 35	17 5	Tiwari <i>et al</i> (1973)
5	Chokla	13 42	17 4	Tiwari <i>et al</i> (1973)
6	Rambouillet × Chokla	13 34	16 9	Tiwari <i>et al</i> (1973)
7	Rambouillet × Malpura	13 34	17 35	Tiwari <i>et al</i> (1973)
8	Rambouillet	13 34	16 9	Tiwari <i>et al</i> (1973)

return basis could be accepted as an indication of pregnancy in 80 to 90% mated ewes. In Jaisalmeri, Sonadi, Pugal Magra and Nali breeds of sheep the duration of oestrus is longer in the first cycle than in the second cycle ranging between 23.2 to 32.2 hrs (Honmode, 1971). Taparia (1972) reported the oestrus duration of 28.5 hrs ranging between 12 to 72 hrs in Sonadi ewes with 88% of total observations in the range of 24 to 36 hrs. Tiwari *et al* (1973), studied the detection of oestrus in Rambouillet, Malpura, Chokla, Jaisalmeri and Rambouillet half breeds. It was 12.24 hrs in a fairly high (36.13%) number of oestrous cycles in all breeds except in Malpura (28%) and Chokla (20%). In general 40 — 50% of the oestruses were in the range of 24.38 hrs duration. The duration of oestrus in Chokla sheep (18.72 hrs) seems to be longer than other breeds.

#### Gestational oestrus in ewes

The occurrence of oestrus during pregnancy is commonly observed in the pregnant ewes. Williams *et al* (1956) reported periods of heat at intervals of

3 to 40 days with a mean of 22 days. These were not restricted to any one part of pregnancy period and these heats were anovular. Hafez (1968) reported that gestational oestrus may occur in pregnant ewes during early stage. It may even occur as late as five days before parturition.

#### Post-partum oestrus

Sahani and Roy (1967) reported the postpartum interval in Bikaneri ewes as 41.6 days (range 17 to 93 days). Nearly 70% of the Bikaneri ewes came in heat between 30 and 50 days after parturition. Taparia (1972) reported the postpartum oestrous interval in Sonadi ewes as 62 days (range, 10 to 80 days).

#### BREEDING SEASONS IN GOATS

In most of the tropical countries oestrous cycles in goats occur throughout the year, while in temperate climate they are seasonably polyoestrous. Bissonette (1941) reported that sexual cycle in goats can be induced following gradual reduction of light exposure and



would terminate earlier than normal following an increase. He also reported that changes in ambient temperature were not a major factor in the control of breeding cycles in goats. Lall (1954) reported that the breeding season of Goats in U.P. (India) extends from May to July, whereas in Haryana it is from March to July. Gupta *et al* (1964) reported that in Black Bengal breed of Goats the frequency of kidding was more in summer months. An analysis of the data pertaining to the occurrence of oestrous cycle in Indian goats, Mishra and Biswas (1966) reported that the highest number of oestrous cycles occurred in October followed by June and the least in March. Roy *et al* (1962) reported that the Jamnapari goats came in heat from July to August. Amble *et al* (1964) observed maximum kidding in Beetal goats from March to April and October to November. Tehbe *et al* (1935) reported that social factors influence the onset of oestrous cycle in goats. It is usually observed that in the presence of male the senses of smell, sight and contact all combine to produce the stimulation of onset of oestrous cycle and ovulation in the doe.

### Oestrous cycle

The average length of oestrous cycle in goats is 20 days (15 to 24 days). In does also, the oestrous cycle has the following phases viz. proestrus is rather indefinite and the onset of oestrus is abrupt. Misra and Biswas (1966) observed the duration of oestrus as 38 hours and that oestrus occurred at any time of the day. Sahani and Roy (1967) reported 30 hours as the duration of oestrus. Cessation of oestrus is gradual. The does in oestrus frequently show a peculiar, continuous bleating and the milk yield may drop. Anorexia and swelling of the vulva are usual symp-

oms of oestrus in does. The tail is shaken continuously during the period. In some animals this period is short when a careful watch is necessary. Sometimes they give very little indication of being in heat. The doe sometimes rubs her neck and body against the male, seek out and follow the male, elevate and shakes the tail. Does usually compete with other females in oestrus to attract the male, even interfering in the male mounting the other female and standing still to receive him (Hafez, 1962 and Mattner *et al*. 1967).

### Duration of oestrus

The average duration of oestrus in goats is about 40 hours (Asdell, 1946). Under Indian conditions the duration of oestrus in goats varies from 20-30 hours (Sane *et al*, 1948). Signs of oestrus are similar to those in sheep, except that bleating is more pronounced and excitement observed to a marked extent. Variable number of desquamated cells in the vaginal smears of goats are observed one to three days after oestrus. Leucocytes and spherical cells are found throughout the period of the cycle, being least when the desquamated cells first appear. The average morning rectal temperature is lowest on the day prior to oestrus and it rises significantly on the day of oestrus (Parker, 1963).

Paul Choudhari (1957) stated that the oestrus period in goats under Indian conditions extends from one to three days and the duration of oestrous cycle varies from 19 to 21 days.

Mishra and Biswas (1966) reported in Deshi goats of Bihar the average duration of heat as  $37.64 \pm 1.13$  hours. Distribution of heats at different times of the day was more or less uniform. Roy *et al* (1962) reported in Jamnapari

goats the duration of oestrous cycle as  $20.6 \pm 2.2$  days

Time of ovulation in the goat is towards the end of oestrus so that if mating is restricted it should be arranged towards the end of oestrus — at least 12 hours after the oestrus is first observed

### BREEDING SEASON IN SOWS

The sows are definite year long breeders like the cow without any manifestation of unduly long anoestrus period during the year. However, marked variations in breeding efficiency are observed from season to season. Zukerman (1953) reported that wild swine have only one sexual season in the year.

#### Oestrous Cycle in sows

The sow is polyoestrus. The length of the oestrous cycle is about 21 days. The duration of oestrus is two to three days. It is shorter in gilts (40 to 46 hours) than in the adult sows.

McKenzie and Miller (1930) observed an average duration of 40-46 hours oestrus in the sow with regular cycles.

Corner (1921), Krallinger (1932) and Asdell (1946) reported that the average duration of oestrous cycle in the domestic sow is 21 days. Oestrus lasts for 2-3 days and may continue up to five days. Burger (1952) reported that the duration of oestrus did not vary with advancing sexual age. There was difference between breeds. The first oestrus periods following parturition and weaning were longer than those preceding conception. Usually oestrus two days after parturition was non-fertile.

Warmick *et al* (1950) observed that the average duration of postpartum oestrus was significantly longer in non-

suckled (60 hours) than in the suckled sows (20 hours).

The period between the end of lactation and onset of first heat is variable. Krallinger (1932) observed an average interval of 7 days in 147 observations. Allen *et al* (1957) observed an average interval of 96 days.

Krallinger *et al* (1932) and Burger (1952) reported that the demands of lactation do not influence interval between the end of lactation and the onset of first heat. Baker, *et al* (1953) observed that suspended suckling reinitiates the cycle.

#### Signs of oestrus

The sow is usually restless. The vulva lips are swollen with congestion and there is reddening of the surrounding region. There is clean fluid mucous discharge. Vaginal wall becomes oedematous during oestrus and epithelial lining thicker and keratinised. The mucus secretion is copious but less viscid. There is congestion of uterus during oestrus. Homosexual tendency is observed.

Rigdy (1967) reported that during oestrus the cervix was found to be rigid and oedematous. It gradually softens as the oedema subsides.

### OESTROUS CYCLE IN THE BITCH

The bitch is classified as monoestrus since she manifests two oestrus periods in a year. April and September is the breeding time of bitches under continental conditions. Asdell (1946) and Hancock and Rowlands (1949) reported that there is no obvious seasonal difference in the incidence of heat in bitches. However, there is difference in the frequency of heat between large and small breeds of dogs. Periodicity is influ-

enced by climatic conditions as is observed in Greenland dogs which usually breed only once a year (Rink, 1877).

### Pro-oestrus

The prooestrus period in bitches lasts for about 8-10 days (average 9 days). It is characterised by swelling and congestion of vulval lips. In about 2-3 days time a clear yellow mucus discharge mixed with blood which is voided from vulva and continues until beginning of oestrus. During prooestrus males are attracted but the bitch will refuse coitus.

### Oestrus

At the onset of oestrus proper, the discharge changes to clear fluid. The oestrus lasts for about 8-10 days (average 9 days). The bitch will accept the male during true oestrus only and the period of intense desire is in the first 2 to 3 days of oestrus period. In bitches the courting period is comparatively longer than other domestic animals. During anoestrus, the vaginal epithelium consists of 2 or 3 layers of columnar cells. During prooestrus and oestrus there is proliferation of the vaginal epithelium which undergoes stratification and in the height of oestrus, sheds its keratinised surface cells. Evans and Cole (1931), Griffiths and Amoroso (1939) observed that bleeding does not cease until after the second day of true oestrus.

Hancock and Rowlands (1949) have reported that the average length of the prooestrus and oestrus periods ranged from 18-28 days in different breeds. The prooestrus or the bleeding phase varied from 5-12 days and the oestrus from 10 to 12 days.

### Vaginal cytology

During proestrus erythrocytes and epithelial cells will be found in the vaginal smear. During anoestrus condi-

tions the vaginal smear will contain epithelial cells and leucocytes which are relatively few. With the onset of proestrus, leucocytes disappear rapidly and there is appearance of the cornified epithelial cells. During oestrus, the smear consists entirely of keratinised epithelial cells and erythrocytes whereas in the later stage of oestrus, leucocytes begin to appear along with small epithelial cells. In the early part of met-oestrus leucocytes are present in large number.

### Changes in the Uterus

During proestrus there is a progressive blood supply to the uterus, with extravasation of erythrocytes on the surface of the endometrium which appear in the vaginal discharge. The glandular size and the activity increases in the latter part of the oestrus and is continued thereafter during pseudo-pregnancy. There is a well marked hypertrophy of the uterine glands and an enlargement of the uterus which reaches its maximum at about 20 days post-oestrus. A month following the oestrus there is regression in the glandular activity and the size of the uterus. This slow regression continues for about two months. During proestrus and oestrus periods the uterus becomes enlarged and turgid. In anoestrus condition, the uterus is characterised by a low columnar or cuboidal epithelium.

### Pseudo pregnancy, (Pseudo-cyesis, Phantom pregnancy):

In bitches, if pregnancy is not established a condition known as pseudo-pregnancy lasting about two months may supervene. During this period, corpora lutea persist with proliferative changes in the uterus and mammary glands. At the end of this period, usu-

ally there is milky-white vaginal discharge which is followed by external bleeding. Mammary activity is marked at the end of pseudo-pregnancy. Milk secretion commences and the bitch prepares bed for the pups as if preparing for whelping and exhibits maternal instincts. The pseudo-pregnancy is followed by anoestrous condition which lasts for about two months or so.

In case of psychic disturbances, Stilboestrol 5 mg or Testosterone 2 to 20 mg, administered i/m daily for a period of about a week may possibly relieve the condition. Mammary tension can be relieved by milking twice or thrice a day. Vinegar may be applied round about the teats for drying.

### OESTROUS CYCLE IN THE CAT

The cat is seasonally polyoestrus with two or three breeding seasons during the year. In the absence of a male, a number of periods would recur during each season. Under continental conditions the breeding season is noticed during the spring and early summer. Most of the cats show oestrus during September and January.

Duration of oestrous cycle in the cat is 14 days and she experiences fortnightly cycles regularly for several months.

#### Pro-oestrus

During this period the female likes the association of the male but does not allow service.

#### Oestrus

During oestrus the cats become playful and excessive rubbing and rolling is noticed. The duration of oestrus in cats is 9-10 days (Roberts, 1956). Oestrus recurs in about 2-3

weeks time (Asdell 1940). Cats that are not bred during 3-4 oestrus periods exhibit oestrus at the next breeding season, usually during late spring and early winter. In the presence of the male, oestrus lasts for about 4 days only as ovulation is induced by coitus. A curious low call, with a different tone accompanied by bending the prepart of the body is a typical symptom of oestrus in the cat. The female assumes a characteristic crouched position resting on chest and forelegs with raised pelvis, elevated tail and treading with hind legs while accepting the male. Oestrus is inhibited during lactation. It recurs within three to four weeks following the weaning of kittens.

#### Changes in Vagina

During anoestrus, the vaginal epithelium has a few layers epithelial cells whereas there is a rapid proliferation of superficial cells which become flattened in prooestrus stages. During oestrus there is rapid growth of the vaginal epithelium having several layers of cornified cells. During the last phase of oestrus there is gradual desquamation of cells resulting in invasion of leucocytes.

Vaginal smears during anoestrus show nucleated epithelial cells which become numerous during prooestrus. During oestrus the vaginal smears show large non-nucleated cornified cells and only a few nucleated cells. During met-oestrus, there is increase in the number of leucocytes.

#### Changes in the uterus

During anoestrus condition the uterine glands are straight and are not extended deep into the endometrium. The size of the glandular and surface epithelial cells is similar. During pro-oestrus the surface epithelial cells are en-

larged. During oestrus, there is no further enlargement of the surface epithelium but the glandular size is progressively increased though the glands are still un-coiled. During the luteal phase there is a marked coiling of the glands. In the absence of ovulation, retrogressive changes start with invasion of leucocytes.

### OVARIAN ACTIVITY IN BOVINES

The right ovary is more active than the left in the cow (Casida *et al* 1935; Clark, 1936; Reece and Turner, 1938; Trimberger, 1948 and Madan & Razdan, 1966). Ahuja *et al* (1961) recorded the frequency of ovulation in Haryana cows as 60.5% and 39.5% from right and left ovaries respectively. Choudhary *et al* (1965) observed in Haryana heifers 70.21% and 29.79% right and left ovulations respectively. Hukeri (1965) in Tharparkar cows recorded 60.4% ovulations from right ovary and 39.6% from the left ovary.

In Murrah buffaloes, the right ovary is more active than the left (Reddy, 1960; Kaikini and Pankey, 1974). Wang (1965) reported that in Nanking buffalo cows, right ovary is more active than the left (1: 0.63). Similar findings in Berari (Nagpuri) buffaloes have been recorded by Kaikini (1971) and Kaikini and Pankey (1971).

development of the Graafian follicle and ovulation is dependent on the gonadotropins secreted by the hypophysis.

In cow and buffalo, normally one ovum is produced, rarely two or more. In the mare normally one and occasionally two or more ova may be liberated. In the ewes, one, two or three ova are produced according to the nutritional status at and before oestrus. Sow produces anything from 6 to 20 ova. In the bitch there is great variation from 1 to 10 or even more.

Age has an effect on the number of ova shed. This is particularly evident in the polyovular species. The size of the first few litters in most of the animals is smaller than the successive ones. The litter size gradually decreases during the declining years of life.

Species in which multiple ovulation is a rule, younger animals produce less number of ova as compared to adults. Genetic make-up also has an influence on the number of ova produced by an individual. In addition to this as stated by Hammond (1927) the plane of nutrition and environmental conditions have a marked influence on the number of ova shed.

rate of ovulation than those fed on low plane of nutrition before flushing. In flushing a high energy or TDN intake is needed but an increased protein is not necessary (Memon *et al* 1969). Young does and older ones have a low ovulation rate. Peak period was in between 3 to 6 years. Cross breeding increases ovulation rate than pure breeding. Shelton (1960) reported that in Angora does the second and third ovulations were more fertile and more ova were released than the first oestrus.

Ovulations were slightly more frequent on the right ovary than the left, i.e. 53 to 55% and 44 to 47% respectively (Hulet *et al* 1962, and Casida *et al* 1966). In single ovulations 62% were on the right ovary. Achuthan Kutty and Raja (1971) reported that 44% ova were found released from the left ovary and 56% from the right ovary.

#### Migration of zygote

In 176 genitalia studied, 41.48% showed trans-uterine migration of embryos (Achuthan Kutty and Raja, 1971). The rate of migration from right horn to the left and left to right were 38.4% and 61.6% respectively. The low rate embryo migration in single ovulation and a high rate upto 60% after double ovulations has been observed. The rate of migration has been reported to be 15.54% in single ovulation as against 88.86% in multiple ovulations (Nair and Raja 1972). Nair and Raja (1973) have also observed that the incidence of trans-uterine migration was 37.35% in goats.

#### POST PARTUM OESTRUS

Following parturition, the female is expected to exhibit oestrus within couple of months. The occurrence of first oestrus after parturition is depen-

dent on a number of factors such as involution of uterus, endocrine constitution, hormonal balance, nutritional status, uterine health and mammary function. After parturition if everything proceeds normally, the uterus must return to its normal position within a specified time. This reposition is physiological.

The first few post-partum heats may be weak and anovular and chances of conception are not certain. It is therefore preferable to breed at heat expressed after complete involution of the uterus for fairly good conception rate.

#### Cow

It is usually observed that the interval from parturition to first oestrus is prolonged in the cows in which suckling is practised as compared to those in which weaning of their calves is done immediately after calving. Silent heats with ovulation have been observed to occur in certain number of cows that are nursing their calves.

Clamohog (1952) reported the average interval between calving and first post-partum oestrus as 116.1 (42-152) days. Mies Filho and Costa Aroeira (1955) reported the average interval between calving and post partum oestrus in Zebu cattle as 87 (12-259) days. Beshlebnov (1956) recorded 28.7% post-partum oestruces of 2971 cows of which 6.5% conceived within 30 days of calving, and concluded that cows of average production should be served at about 60 days after calving and highly productive cows between 60 and 80 days. Politov (1957) found the average interval between calving and service as 72.1 days and that of calving and conception 91 days. Buch (1957) reported the average post-partum interval in Holstein Friesian as 33 days

and for complete involution of uterus 47 days. He observed that the first oestrus was shortest in summer and longest in winter. Kohli and Suri (1957) reported the first post partum oestrus in Harijana cows at  $228.0 \pm 3.9$  days. They observed that the season significantly affects the post-partum interval to first oestrus as a result cows calving in the month of April to July which have a longer interval than those calving in other months of the year. They also observed that age has a significant influence on the interval as the older the animal, longer the interval. The mean length of post-partum interval to conception in Tharparkar Cows was  $131 \pm 79.8$  days. The post-partum interval was significantly higher after first calving than after subsequent calvings. But the season of calving and level of milk production had no significant effect (Prasad, 1958). The average period of post-partum oestrus in Holstein cows is 76.3 days and post-partum fertile oestrus 115.4 days (Higaki *et al*, 1959). Biswal and Rao (1960) recorded in Red Sindhi cows, the occurrence of first heat after first, second and third calvings was 114,

106 and 107 days respectively, the overall being 154 days. Luktuke and Subramanian (1961) recorded post-partum oestrus interval in Harijana cows as 84.56 (11-390) days with an average of 135.71 days of post-partum conception interval resulting in 47.34% conceptions. Ahuja *et al* (1961) reported in Harijana cows, the first post-partum oestrus on an average as  $80.7 \pm 15.8$  days. Norwood (1963) reported the average interval from parturition to first insemination to conception and to uterine involution for cows inseminated at first post-partum oestrus as 39.75, 68.84 and 41.24 days respectively. Hukeri (1965) reported that the average interval of the first post-partum oestrus and the first post-partum fertile oestrus in Tharparkar cows as  $71.04 \pm 6.1$  and  $79.0 \pm 12.2$  days respectively. He further observed that 66.66% of first post-partum oestruses are ovulatory with a conception rate of 34.78% indicating that the majority of the first post-partum oestruses were ovulatory and fertile. Similar findings are reported in Dangi cows by Purbey (1965), (Table 25).

Table 25  
MANIFESTATION OF THE FIRST POST-PARTUM OESTRUS IN THE COW

Breed	Post-partum oestrus interval in days	Reference
Red Sindhi	$120.0 \pm 10.61$	Sane <i>et al</i> (1968)
Sahiwal	$39.33 \pm 1.45$	Kadu and Kaikini (1975)
Gir	$146.0 \pm 5.1$	Phatak (1965)
Gir	$136.0 \pm 19.8$	Sane <i>et al</i> (1968)
Gir	$179.78 \pm 6.9$	Ponkshe (1969)
Gir	$158.41 \pm 10.94$	Dange (1969)
Gir	187.5	Mokashi (1972)
Rathi	$117.0 \pm 5.34$	Sonawane (1969)
Nimar	143.87	Sane <i>et al</i> (1956)
Khillar	$158.88 \pm 4.16$	Tatke (1967)

## Buffaloes

Observations on the post partum oestrus in buffaloes are more or less similar to those in cows. The first post partum oestrus in Azarbaizan buffaloes occurs in 16.22 days and subsequent oestruses after 15 to 30 days (Basirov, 1964). El Dessouky and Rakha (1964) reported that first post partum oestrus in Egyptian buffaloes was manifested at  $134.5 \pm 14.62$  days. Kohli and Malik (1960) reported in Murrah buffaloes a mean service period of  $201.6 \pm 11.31$  days. Minimum service period was recorded for spring calves and maximum for those calving during summer. However, seasons had no significant influence on service period. El Sheikh (1967) reported in Egyptian buffaloes an average service period of  $165.3 \pm 3.2$  days. Buffaloes calving during July to November had shorter service periods than those calving during the rest of the year. The longest service periods were for buffaloes that calved in March (213.57 days) and the shortest were for those calving in October (129.71 days). Venkayya and Anantkrishnan (1966) reported that the mean service period in Murrah buffaloes was 173.9 days and monsoon calvers had a significantly shorter service period as compared to winter and summer seasons. Shalish (1958) reported in Egyptian buffaloes that the average interval between calving and first oestrus was  $141.11 \pm 6.1$  days. Sex of the calf had a significant influence as also the month and sequence of calving on the length of service period. Dominiques (1961) reported that in Brazil the first oestrus in buffaloes after parturition occurred from 15 to 90 days. Sane *et al* (1968) reported the interval between calving and first heat in Surti and Murrah buffaloes as  $90.0 \pm 8.57$  and  $111.21 \pm 12.33$  days while the interval between calving and

first fertile heat in Surti Buffaloes was  $120.0 \pm 12.44$  days. Pargaonkar and Kaikini (1968) and Pargaonkar (1969) recorded post partum oestrus in Nagpuri (Berari) buffaloes occurring on an average 55.10 days after calving.

## Mare (Foal heat)

The first post partum oestrus in the mare usually occurs between 7 to 14 days. If mares are bred at this time the conception rate is low. In some mares oestrus occurs as late as 45 days after parturition. This is likely to have preceded by a quiet ovulation.

Britton and Howell (1945) reported that in the mare the interval between post partum oestrus and the subsequent oestrus may be affected by milk production. Jennings (1950) opined that breeding at foal heat may cause abortions, stillbirths and retained afterbirths. This is due to the likely introduction of infection when involution of uterus is incomplete — a stage at which uterine epithelium is not completely restored (Nishikawa and Hafez, 1974). Loy and Swan (1966) observed that the so called 30 day heat or second heat after foaling occurred regularly in the thorough bred mares.

## Ewes

In most of the ewes there is a definite breeding season and lambing occurs once a year. As a result of this period required for involution of the uterus and occurrence of first post partum oestrus is not much of practical importance. In consequence of this ewes are seldom bred during the period when they are nursing the young ones. They are usually bred months after the lambs are weaned. The prolonged interval allows sufficient time for complete involution of the uterus before the com-



mencement of the next breeding season. In breeds of sheep which produce two crops of lambs a year, lactational dioestrus usually delays the return of oestrus immediately after parturition.

### Sows

The process of involution in the sow is more or less similar to that in the cow. In the sow the first oestrus after farrowing usually occurs within one to three days. This oestrus is usually unaccompanied by ovulation and the sows if bred at this time the conception rate is usually poor since the follicles remain immature (Anderson, 1974). The production of milk lengthens the interval from parturition to first oestrus and ovulation. If piglets are allowed to suckle the sows for a full period of two months, sows may not come into oestrus even after 48 days of lactation. Most of the sows come into oestrus in about a week's time after the piglets are weaned. The interval from weaning to onset of oestrus is shortened and less variable as the lactation period progresses. Pituitary FSH activity is high during early and late lactation while as LH activity remains low throughout lactation. The condition of lactational anoestrus may be a period of depressed FSH release and reduced LH synthesis (Anderson, 1974).

Magnani and Gassati (1966) reported in crossbred sows (large White Spotted Poland China X Chesterwhite) in Italy that the interval between farrowing to first oestrus varied between 58.94 days in May to 64.60 days for those in January. It was significantly shorter for May and June than for other months.

### FACTORS INFLUENCING OESTROUS CYCLE

The effect of environmental conditions on pituitary activity varies from

species to species, as a result of which changes in breeding season are noticed. In the cow, the pituitary response is more or less uniform due to which regular cycles occur throughout the period of the year, with greater or lesser intensity. In the ewe, the response is restricted to a particular period of the year when environmental conditions are favourable and feed is in plenty. In the external control of oestrous cycle there is predominant influence of three factors — (1) Environmental temperature (2) Nutrition and (3) Light. In the birds, proximity of males is also an important factor, likewise in farm animals. The vicinity of a male is equally important in initiation and maintenance of oestrus and oestrous cycles.

#### 1. Environmental temperature

Extreme weather conditions such as cold or heat depress the sexual activity and usually less number of conceptions occur when the temperature is severe. Decreased thyroid activity during severe summer conditions may indirectly reduce the reproductive efficiency. Dutta and Bush (1955) observed that the temperature has some influence on the time of onset of breeding season in sheep. Allisten and Albert (1961) observed that high temperature may lower the number of young produced by a female. There is low rate of fertilization and a higher rate of embryonic death though oestrus and ovulation rate was not affected.

#### 2. Nutrition

It is evident that low nutritional status for a prolonged period or continued starvation delays puberty in young animals. Diet deficient in Vit. A and phosphorus content may delay the

onset of breeding season in ewes. In adults this will either lead to disturbances or suppression of the normal rhythm. In ewes considerable influence of nutritional status is observed and better lamb crop is obtained if the plane of nutrition is optimum before and during oestrus. Oestrogenic substances in plants during season also influence oestrus and oestrous cycles.

Year to year variations in the onset of breeding season have been observed in the same ewes but breed wise differences in the onset of oestrus are not obviously noticed. This is suggestive of individual variation but if selection is to be based on this trait, the repeatability coefficient for the initiation of breeding season is so low that the progress will be very slow.

In general, it is observed that pronounced under feeding causes delayed maturity in heifers and in cows prevents maturation of Graafian follicles. This is more marked in the ovaries of younger animals. Sane *et al* (1967) observed plenty of heifers and cows with such a condition in heavy rainfall areas and hilly tracts of Maharashtra due to prolonged exposure to inclement weather and poor feeding. In areas with cobalt deficiency, the onset of oestrus and ovulation in sheep in the beginning of rainy season is often delayed. Dunlop (1944) has shown good results by feeding cobalt under similar condition. Quinlan *et al* (1941) reported that in the mineral deficient areas the addition of phosphates in the form of bone meal increases the percentage of heifers which come on heat and the calving percentage from 56 to 87. In sheep the effect of food supply on the number of follicles that mature at each oestrous period is comparatively more marked

and the number of lambs born is very closely related to the number of ova shed. The amount and type of food affects fertility in the same animal from season to season. Sheep in the mountainous regions never produce more than one lamb whereas the same sheep when brought down on the plains frequently give twins.

### 3 Light

Oestrous cycle is externally controlled by the duration and intensity of daylight to which the animal is exposed. Rowan (1938) has demonstrated this effect in birds and ferrets. Bedfords and Marshall (1942) demonstrated the effect of transferring seasonally polyoestrous animals from southern to northern hemispheres and vice versa and showed that they do breed during the corresponding winter or autumn at the new place, however this reversal effect was not immediate in all cases. In cows suppressive effects of oestrous cycles have been observed during the winter months and better effects on breeding in spring season. Mercier and Salisbury (1947) observed that the fertility is progressive as the daylight is lengthened. Burkhardt (1947) observed that the mare responds to supplements of artificial light by breeding earlier in the spring. Yeates (1949) demonstrated that sheep responded to decreasing day light length in summer and increasing amounts in winter. Sheep are known as 'short day breeders'. Mares and Poultry responds to increasing day lengths and are therefore known as long day breeders.

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# Chapter 18

## Corpus Luteum

### Cyclical development in the cow

After ovulation the follicular wall collapses. At this time there is an extravasation of blood and this together with the cell debris and leucocytes form a tenacious coagulum in the empty space (crater) of the follicle which further protrudes on the ovarian surface. This is called corpus rubrum.

The corpus luteum develops from the granulosa and theca cells may also contribute towards its formation. The development of the luteal tissue takes place under the influence of the luteinising hormone of the anterior pituitary. The active glandular portion is developed from the granulosa cells and the connective tissue and vascular tissue from the theca cells. The blood clot formed in the early stages due to extravasation of blood is gradually resorbed as the luteinisation progresses and ultimately the entire cavity of the ruptured follicle is occupied by the active corpus luteum.

In very early stages the corpus luteum is termed as corpus haemorrhagicum which protrudes above the surface of the ovary and has a central depression. In about 24 hours time the mass may come up to the surface of the ovary or is elevated slightly above the level of

its surrounding surface. The portion that protrudes above the surface of the ovary may have a depression in the centre which looks like a crater in the latter stages of development. This depression marks the point of rupture of the former follicle. The corpus haemorrhagicum stage lasts for about 2 to 3 days. The shape of corpus haemorrhagicum is usually oblong depending upon its place in the ovary and the pressure caused either by a nearby follicle or remnant of the previous corpora lutea. The mass is soft and fleshy in consistency. The size of the corpus luteum is dependent on the breed and also the pituitary activity. It is about 1 cm in diameter and may vary from 0.8 to 1.2 cm. During the first two or three days the colour of the corpus luteum changes from chocolate red to light liver colour. The average weight at this stage is from 4.1 gm to 7.4 gm (Benesch & Wright 1951).

It increases in size and weight rapidly during the first four days and reaches  $\frac{3}{4}$ th of its normal size during this period.

In about a week's time, the corpus luteum develops rapidly due to the proliferation and hypertrophy of the luteal cells and its colour gradually



changes to brownish pink or pink-brown. At one week, the corpus luteum has an approximate size of 1.5 to 2.0 cm. The average weight at this stage is 4.5 to 6 gms.

In the cow, the corpus luteum usually reaches its maximum weight at about the 12th day with an approximate diameter of 2 to 2.5 cm. Hafez (1962) reported that the growth period of corpus luteum extends a little longer than half the total length of the oestrous cycle. Hammond (1927) observed that the corpus luteum attains its maximum growth by about the 8th day of the cycle. Laing (1955) reported that the diameter of the corpus luteum increases from about 5 mm immediately after ovulation to 20 to 25 mm at mid-cycle. It then gradually declines from 14 days onwards and at the end of the cycle, its size is reduced to 10 mm in diameter on account of the regression. Cole and Cupps (1943) reported that the young corpus luteum measures 6 to 8 mm, by 8th day the size increases to 18 to 20 mm, the maximum size of the mature corpus luteum being 20-25 mm. Zemjanis (1962) reported that the average diameter of corpus luteum between 8 to 17 days varies from 10 mm to 20 mm. Hafez (1962) reported that in the non-pregnant cows, regression of corpus luteum commences from 14th to 15th day after oestrus and in the early stages the regression proceeds very rapidly reducing the size of the corpus luteum to half its normal size within 36 hours.

The mature corpus luteum of the cow projects well above the surface of the ovary and the projection is usually spherical with a distinct table like surface on the top with a crater in the centre. In well developed corpora lutea, there is a distinct neck formation which can be easily felt on palpation per rec-

tum. It may occupy more than half the ovary. In certain cases it may assume bigger size than the ovary particularly between the 8th and 12th day. During this period, the colour changes to light yellow and by about the 14th day it assumes a distinct yellow colour. The yellow colouration is due to lipoid granules. The enlarged granulosa cells in the cow and mare contain a yellow pigment known as lutein. The pigment gives yellow colour to the corpus and hence it is popularly known as the "yellow body" in the cow and the mare. The corpus luteum maintains its maximum size upto the 16th day and thereafter slowly regresses. The feel of the corpus luteum from 8th to 16th day is very firm. Thereafter it gradually becomes harder. On regression it shrinks in size, becomes white in colour; gets deeply embedded in the ovarian tissue and is termed 'Corpus albicans'. It takes a long time for complete regression.

Hammond (1927) reported that in the cow the corpus luteum during early stages is pale but soon changes to creamy and then bright yellow turning to orange red, finally assuming bright scarlet or brown colour.

### Function of the Corpus Luteum

The function of the corpus luteum is to secrete progesterone. The level of progesterone gradually increases reaching its peak between the 8th and the 12th day and further it remains more or less constant upto the 18th day, declining rapidly thereafter.

### Corpus Luteum in the Buffalo

Sane *et al* (1964 and 1965) reported that the fresh corpus luteum of Murrah and Jaffri Buffalo is deep red in colour which changes to bluish red and then to

dirty grey on regression. In a study of over 1700 ovaries it was observed that the colour of the buffalo corpus luteum distinctly keeps to dark bluish red for major number of days of the oestrous cycle and does not become lighter until the approach of the subsequent oestrus

### **Cyclical development of the corpus luteum in the mare**

In the mare, profuse bleeding occurs after ovulation and the corpus luteum is haemorrhagic for the first 2 or 3 days. Formation of corpus luteum is very rapid. Hamilton and Day (1945) reported that within about 8 hours after ovulation the follicular cavity is filled up with a blood clot which remains soft and pliable for about 10 hours and becomes plum like in consistency after about 24 to 30 hours. It gradually becomes firmer and cannot be easily located on palpation and by about 4 or 5 days, it is no longer palpable. In the mare, palpation of corpus luteum becomes difficult on account of the dense tunica albuginea and more, since it is usually centrally located in the ovulation fossa. However, the ovary with the corpus luteum is normally about twice the size of the inactive ovary. Harrison (1946) reported that immediately after ovulation, within 18 to 24 hours there is invasion of the granulosa by the theca interna cells, disintegration of the granulosa cells and commencement of luteinisation. During the next 24 hours, the theca-interna cells invade the collapsed follicle which gets the appearance of trabeculae. The trabeculae have certain distinguished features as a result of which it can be differentiated from corpus luteum in other species. In about four days after ovulation, the follicular cavity is practically filled by the corpus luteum. The corpus luteum develops to its maximum size at about the 10th day. At this time

the fertilised ovum has already entered the uterine cavity. By about 14th day the corpus luteum reaches its maximum size. It is somewhat triangular in shape with its apex towards the ovulation fossa. Between the 10th and the 14th day, it reaches maximum width of about 3 cm. Its functional activity continues till about 17th day. If fertilisation has not occurred, the corpus luteum ceases to function. It remains in the ovary as a distinct structure for 2 to 3 cycles. During early stages, the corpus luteum is of a buff colour and gradually becomes darker as the cycle progress. Hammond (1941) and Harrison (1946) reported that as the cycle progresses, the corpus haemorrhagicum gradually changes to brownish colour which later becomes dark during regression.

Degeneration of the corpus luteum occurs after about 7 weeks.

### **Corpus Luteum in the ewe**

The corpus luteum in the ewe, resembles that of the cow in its development, reaching its maximum size in about a week, with an approximate diameter of one cm. During active stage it is pink in colour which gradually changes and becomes yellow towards the regression phase when it degenerates. Restall (1964) reported that 2 days after ovulation, the corpora lutea were deep red in colour with a progressive change to pale and later to yellow on or about the 18th day.

### **Corpus luteum in the Sow**

In the sow, the histological changes in the development of the corpus luteum are more or less similar to those in the cow and the ewe. On rupture of the follicles after ovulation, haemorrhage occurs in the central cavity. The

luteal tissue is formed mainly by the granulosa cells and to some extent from theca interna cells. The corpora lutea reach their maximum size within 8 to 10 days. They are hemi-spherical in shape and have a diameter of about 1 cm. According to Hafez (1962) and Day (1962), the corpora lutea of swine vary from flesh red to pink in colour and the corpora albicans are light yellow. Zaki and Hadi (1967) have mentioned that the colour of corpora lutea gradually changes from yellow to pale brown during regression. In the pig the corpora lutea are light grey, tinged with pink and change to yellow colour on regression. It is a distinguishing feature in this species that even on regression of the corpus luteum, the pigment does not disappear by resorption but remains in the regressing tissue. Corner (1921) reported that in a non-pregnant sow, the corpora lutea attain their full size of 8 to 9 mm. in diameter in about a weeks time after ovulation. Regression usually begins on 15th day. However, the next oestrus does not occur until 21 days. Day (1962) observed that in case a sow has not settled to service, regression of the corpora lutea commences from 14th to 16th day of the oestrous cycle with reduction in size to the extent of 50% during oestrus — rate being maximum during ovulation.

### **Corpus luteum in the bitch**

In the bitch, corpora lutea form a soft structure made of loose columns mainly of granulosa cells. They become firm on or about the 18th day and remain functional for about 30 days after the cessation of oestrus. Mulligan (1942) reported that the corpus luteum is matured in the bitch by about the 13th day of metaoestrus. Thereafter there is slow regression. Evans and Cole (1931) and Mulligan (1942) reported that in

the non-pregnant bitch regression of the corpora lutea begins about a month after oestrus and the following two months are occupied in slow regression. This phase, is associated with a state of pseudo-pregnancy.

The mature corpus luteum has an approximate diameter of 5 to 6 mm and appears as a firm reddish yellow nodule.

### **Corpus luteum in the cat**

In the cat there is no haemorrhage at ovulation. The walls are deeply folded and there is a secretion of secondary liquor folliculi for about 24 hours, after which the granulosa cells become active. The lutein cells are mainly formed from the granulosa layer. In the cat the corpora lutea reach their maximum size within about 10 to 16 days after coitus. Dawson (1941) reported that in the cats, gradual regression of the corpus luteum during pregnancy sets in after 20 days of gestation. Roberts (1956) mentioned that in the cat the sterile service is usually followed by pseudo-pregnancy which lasts for about 36 days though the corpus luteum regression commences from 20 days onwards. Manwell and Wickins (1928) reported that no corpora lutea were found in the female cats which had not been mated and pseudo-pregnancy was absent in such cases. This is on account of the fact that in unmated cats ovulations and formation of corpora lutea do not occur.

### **CORPUS LUTEUM OF PREGNANCY**

If the pregnancy is established, the corpus luteum continues to function and it does not regress as during the normal cycles. The corpus luteum of pregnancy has a firm consistency and is well embedded in the stroma of the ovary. The activity of the cyclical corpus luteum is

dirty grey on regression. In a study of over 1700 ovaries it was observed that the colour of the buffalo corpus luteum distinctly keeps to dark bluish red for major number of days of the oestrous cycle and does not become lighter until the approach of the subsequent oestrus.

### **Cyclical development of the corpus luteum in the mare**

In the mare, profuse bleeding occurs after ovulation and the corpus luteum is haemorrhagic for the first 2 or 3 days. Formation of corpus luteum is very rapid. Hamilton and Day (1945) reported that within about 8 hours after ovulation the follicular cavity is filled up with a blood clot which remains soft and pliable for about 10 hours and becomes plum like in consistency after about 24 to 30 hours. It gradually becomes firmer and cannot be easily located on palpation and by about 4 or 5 days, it is no longer palpable. In the mare, palpation of corpus luteum becomes difficult on account of the dense tunica albuginea and more, since it is usually centrally located in the ovulation fossa. However, the ovary with the corpus luteum is normally about twice the size of the inactive ovary. Harrison (1946) reported that immediately after ovulation, within 18 to 24 hours there is invasion of the granulosa by the theca interna cells, disintegration of the granulosa cells and commencement of luteinisation. During the next 24 hours, the theca-interna cells invade the collapsed follicle which gets the appearance of trabeculae. The trabeculae have certain distinguished features as a result of which it can be differentiated from corpus luteum in other species. In about four days after ovulation, the follicular cavity is practically filled by the corpus luteum. The corpus luteum develops to its maximum size at about the 10th day. At this time

the fertilised ovum has already entered the uterine cavity. By about 14th day the corpus luteum reaches its maximum size. It is somewhat triangular in shape with its apex towards the ovulation fossa. Between the 10th and the 14th day, it reaches maximum width of about 3 cm. Its functional activity continues till about 17th day. If fertilisation has not occurred, the corpus luteum ceases to function. It remains in the ovary as a distinct structure for 2 to 3 cycles. During early stages, the corpus luteum is of a buff colour and gradually becomes darker as the cycle progresses. Hammond (1941) and Harrison (1946) reported that as the cycle progresses, the corpus haemorrhagicum gradually changes to brownish colour which later becomes dark during regression.

Degeneration of the corpus luteum occurs after about 7 weeks.

### **Corpus Luteum in the ewe**

The corpus luteum in the ewe, resembles that of the cow in its development, reaching its maximum size in about a week, with an approximate diameter of one cm. During active stage it is pink in colour which gradually changes and becomes yellow towards the regression phase when it degenerates. Restall (1964) reported that 2 days after ovulation, the corpora lutea were deep red in colour with a progressive change to pale and later to yellow on or about the 18th day.

### **Corpus luteum in the Sow**

In the sow, the histological changes in the development of the corpus luteum are more or less similar to those in the cow and the ewe. On rupture of the follicles after ovulation, haemorrhage occurs in the central cavity. The

luteal tissue is formed mainly by the granulosa cells and to some extent from theca interna cells. The corpora lutea reach their maximum size within 8 to 10 days. They are hemi-spherical in shape and have a diameter of about 1 cm. According to Hafez (1962) and Day (1962), the corpora lutea of swine vary from flesh red to pink in colour and the corpora albicans are light yellow. Zaki and Hadi (1967) have mentioned that the colour of corpora lutea gradually changes from yellow to pale brown during regression. In the pig the corpora lutea are light grey, tinged with pink and change to yellow colour on regression. It is a distinguishing feature in this species that even on regression of the corpus luteum, the pigment does not disappear by resorption but remains in the regressing tissue. Corner (1921) reported that in a non-pregnant sow, the corpora lutea attain their full size of 8 to 9 mm. in diameter in about a weeks time after ovulation. Regression usually begins on 15th day. However, the next oestrus does not occur until 21 days. Day (1962) observed that in case a sow has not settled to service, regression of the corpora lutea commences from 14th to 16th day of the oestrous cycle with reduction in size to the extent of 50% during oestrus — rate being maximum during ovulation.

#### Corpus luteum in the bitch

In the bitch, corpora lutea form a soft structure made of loose columns mainly of granulosa cells. They become firm on or about the 18th day and remain functional for about 30 days after the cessation of oestrus. Mulligan (1942) reported that the corpus luteum is matured in the bitch by about the 13th day of metoestrus. Thereafter there is slow regression. Evans and Cole (1931) and Mulligan (1942) reported that in

the non-pregnant bitch regression of the corpora lutea begins about a month after oestrus and the following two months are occupied in slow regression. This phase, is associated with a state of pseudo-pregnancy.

The mature corpus luteum has an approximate diameter of 5 to 6 mm and appears as a firm reddish yellow nodule.

#### Corpus luteum in the cat

In the cat there is no haemorrhage at ovulation. The walls are deeply folded and there is a secretion of secondary liquor folliculi for about 24 hours, after which the granulosa cells become active. The lutein cells are mainly formed from the granulosa layer. In the cat the corpora lutea reach their maximum size within about 10 to 16 days after coitus. Dawson (1941) reported that in the cats, gradual regression of the corpus luteum during pregnancy sets in after 20 days of gestation. Roberts (1956) mentioned that in the cat the sterile service is usually followed by pseudo-pregnancy which lasts for about 36 days though the corpus luteum regression commences from 20 days onwards. Manwell and Wickins (1928) reported that no corpora lutea were found in the female cats which had not been mated and pseudo-pregnancy was absent in such cases. This is on account of the fact that in unmated cats ovulations and formation of corpora lutea do not occur.

#### CORPUS LUTEUM OF PREGNANCY

If the pregnancy is established, the corpus luteum continues to function and it does not regress as during the normal cycles. The corpus luteum of pregnancy has a firm consistency and is well embedded in the stroma of the ovary. The activity of the cyclical corpus luteum is

sufficient to maintain the uterus in an optimum condition for implantation. This is evident from the fact that if the corpus luteum is enucleated during early stage, implantation does not occur.

In the cow, ewe and the sow, if conception has occurred, the corpus luteum develops till mid cycle, as during normal cycle. Thereafter, there is no decline in size and activity, and it continues to secrete progesterone actively and approximately keep up to the same size until the end of gestation except in the mare. On account of effect of the progesterone produced by the corpus luteum of pregnancy, oestrus does not occur in the normal course during gestation, though oestrogen is produced in abundance chiefly from placenta during gestation. Regression occurs before parturition. Dawson (1941, 1946) reported that in horse, cat and man, regression of the corpus luteum, starts before the end of the gestation period.

In the cow, the size, shape, and weight of the corpus luteum during pregnancy more or less remains constant 5 to 6.5 gm — range 3 to 9 gm (Roberts 1970). The removal of corpus luteum in the cow during early gestation results in termination of the pregnancy, followed by abortion (Sartons 1949). Hammond (1927) reported that in the corpus luteum of pregnancy in the cow no marked change is usually noticed with regard to colour and size as comparable to the corpus luteum of mid cycle.

In the mare, the corpus luteum develops during the cycle at which conception has taken place. It does not continue its activity as corpus luteum of pregnancy but begins to regress at the end of the first month of pregnancy. This is replaced by a new crop of corpora lutea which have their origin from a new wave of follicular development. The

new follicles start appearing at about the 23rd day of pregnancy. The follicle formation is rapid and several follicles grow at one and the same time during the second and third month of pregnancy, when there is an abundance of gonadotropic hormones in the blood. Cole *et al* (1931) and Amoroso *et al* (1948) reported that the new crop of corpora lutea remain functional until about the 150th day of gestation. By 200th day of gestation the placenta actively secretes progesterone and removal of the corpus luteum at this stage does not lead to abortion.

Cole and Miller (1935) reported that in the ewe the corpus luteum of pregnancy persists until parturition. Grant (1936) reported that though corpus luteum of pregnancy remains until parturition, atrophy usually starts 2 to 3 weeks prior to it. It appears from the work of Cassida & Warwick (1945) that at the end of the second month, pregnancy in the ewe may continue even in the absence of corpus luteum.

In goats total removal of the ovary or of the corpus luteum alone, during the middle or late phase of gestation, usually results in abortion.

Corner (1921) reported that in the sow rapid degeneration of the corpus luteum occurs at parturition. In the bitch, corpus luteum is necessary for nidation and also for maintenance of pregnancy. In pseudo pregnancy of bitches however, the regression of the corpus luteum commences earlier as compared to that of pregnancy. Mulligan (1912) reported that in the pregnant bitch the morphological structure of the corpora lutea is maintained till the end of gestation period. Brambell (1936) reported that in the bitch the functional activity of the corpora lutea is upto

mid-gestation and thereafter it is followed by slow regression.

Gross (1936) reported that if the ovaries are removed in the pregnant cat upto 46 days of gestation, it results in abortion but if oophorectomy is performed at 40 days or later, the foetuses are carried to full term. Dawson (1941) reported that in the cat gradual regression of the corpus luteum during pregnancy sets in after 20 days.

### ENUCLEATION OF CORPUS LUTEUM

Cattle breeders are always keen to have their stock bred in good time to reduce the inter-calving period. But innumerable difficulties are always experienced in the maintenance of good fertility. Factors responsible for subfertility and infertility are innumerable and in a number of instances the assessment becomes difficult when apparently everything appears to be normal. If after parturition, the first oestrus is not exhibited by the cow or buffalo within a stipulated period of 2 to 3 months, the farmer gets worried and in the absence of any other clinical evidence except the anoestrous condition, the Veterinarian is also confused with regard to diagnosis and remedial measures.

On the assumption that retention of corpus luteum in the ovary is responsible for the suppression of oestrus, the veterinarian is tempted to enucleate corpus luteum in cows and buffaloes in the absence of any other effective measures to induce oestrus and ovulation.

Veterinarians all over the world since 1950 adopted this method of enucleation of corpus luteum as a treatment to alter the hormonal balance and effectively bring about changes to combat various reproductive disorders. Based on

the personal experience of the authors and available literature on the subject the advisability of such a treatment is discussed.

Due to wide variations in the climatic conditions and plane of nutrition, a large number of animals are annually reported to the Veterinarians either as subfertile or infertile and in the absence of any other clinical evidence except that there is persistent corpus luteum in the ovary, large number of Veterinarians do enucleate the corpus luteum to bring the animal to heat. Results however are not very encouraging and in the absence of the difficulties of follow up in such cases nothing much is gained except that the enucleation is done. Authentic records on such type of work are very few.

Roberts (1956) mentioned that anoestrus condition may be expressed in two ways viz. (1) presence of a hard corpus luteum or corpora lutea in one or both the ovaries, (2) Cows having small inactive ovaries with a cheesy feel and no functional corpus luteum palpable per rectum.

Great variations are usually noticed in the size and consistency of the corpus luteum depending on the stage of oestrus cycle and pregnancy. A Veterinarian not only should observe large amount of slaughter house material to get himself acquainted with the various types of corpora lutea, their location, size, consistency and the depth to which it is embedded in the ovary.

Herrick (1952) reported his observations on 36 cases of enucleation out of which oestrus occurred in 29 cases in two to seven days period.

Neilson *et al* (1954) reported that heat was induced in 5% cows within 56 hours and the conception rate of 50%

was obtained on breeding these cows on induced heats Hancock (1948) reported his observations on seventy cows that in 35 cows oestrus occurred within two to seven days on enucleation of corpus luteum Ten cows of these became pregnant to service on induced heat Sohoni (1967) observed that in buffaloes heat is induced in 4 to 7 days after enucleation of corpus luteum in about seventy five per cent of the cases Haberg and Fodgeland (1952) reported on their experiment that 59.9%, cows came on heat within eight days after enucleation Jacobson and Teige (1956) investigated 2746 enucleation of corpus luteum from cows and heifers with clinically normal genitals They divided the material in two groups (1) Animals enucleated once (2) Animals in which enucleation of corpus luteum was done on two or more occasions

The greatest number of animals came in heat on the 4th day of enucleation while the conception results have shown higher tendency of success on induced heats occurring on the third day Heats expressed on the seventh day of enucleation of corpus luteum showed better conception rates than for heats in first six days

Amongst animals previously inseminated oestrus was induced on enucleation of corpus luteum in 93.4% Amongst the animals not previously inseminated oestrus was induced in 87.7%

During periods 17, 8-21, 28-29, 29-50 days after enucleation oestrus appeared on an average 62.5%, 9.7%, 17.7% and 8.2%, respectively

Subfertility or infertility conditions in which enucleation of corpus luteum is practised with a slender hope that the return of animal to oestrus may

cause recovery In view of this, indications and contra indications are discussed as under

### Contra Indications

1 *To induce heat in anoestrous conditions in cows and buffaloes* A number of cows and buffaloes are usually brought to the A.I. centres or veterinary hospitals with the history that the animal has not shown the first oestrus even 4 to 6 months after parturition In buffaloes it may even extend from 8 to 12 months In the absence of any other symptoms Veterinarians do feel to enucleate corpus luteum in such a condition Success is however dependent on the type of corpus luteum its consistency and location

If the corpus luteum is functional, it is easier to enucleate But if it is hard and well embedded in the ovary it becomes very difficult to dislodge On enucleation heat may be induced in certain number of animals depending on the type of corpus luteum and pituitary response When a functional corpus luteum is properly enucleated, the level of progesterone is suppressed and the anterior pituitary is jolted to release FSH which in turn induces the follicular development However, it is not very certain if such an induced heat would be ovulatory It should be remembered that heat can be induced in ovariectomised rats without any relation to oestrus phenomenon On complete enucleation of a functional corpus luteum it takes about 3 days for heat to occur The time may vary from case to case

When subfertility due to anoestrus condition becomes a herd problem it is risky to apply the enucleation measure on a mass scale which may prove



dangerous. It is always better to remove the cause rather than to attempt to enucleate corpus luteum.

## 2. *Pyometra*

In this condition the corpus luteum usually persists and there is great temperature for its enucleation. By removing corpus luteum the animal may come to oestrus leading to relaxation of cervix and consequent stimuli to uterus resulting in expulsion of pus. This occurs due to the influence of naturally occurring oestrogen due to induced heat. But the duration of heat is limited from 12 to 24 hours only and there after owing to the formation of corpus luteum the progesterone activity produces contrary effects leading to the quiescent state of uterus and closure of the cervix. The effect of the naturally occurring oestrogen is only for the duration of few hours to a day and in case of pyometra it is necessary to have the effect of oestrogen for a longer duration from a few days to a week so that the foetid material is properly drained and repairs to endometrium also ensured in good time. It is therefore advisable that in such a condition instead of removing corpus luteum and bringing the animal to induced heat, it is better not to enucleate but to make use of synthetic oestrogens for prolonged effects. Moreover, in infective conditions of uteri and fallopian tubes it is not at all advisable to enucleate corpus luteum for fear of spread of infection.

## 3. *Mummification*

In such a condition, if corpus luteum is enucleated similar results as described above under pyometra will supervene. It is therefore not advisable to enucleate corpus luteum in such a con-

dition for expulsion of the mummified foetus. A prolonged stimulus to uterus and cervix is essential and as such it is advisable to administer repeat doses of synthetic oestrogen. It takes long for the mummified foetus to be expelled.

## 4. *Maceration of the foetus*

It is not at all advisable to enucleate corpus luteum although in absence of any other measures veterinarians do resort to enucleate corpus luteum. It is risky to do so on account of infective condition of the uterus and there is every possibility for the spread of infection. It is therefore advisable to use repeated doses of synthetic oestrogens in addition to antibiotics rather than to enucleate corpus luteum.

## 5. *In suspected cases of early embryonic deaths*

In such a condition it is doubtful, if induced heat due to enucleation of corpus luteum can prove effective in restoring the female to normal reproductive efficiency and to bear the conceptus. Attempt should be made to eliminate the causative factors.

## 6. *Infertility Treatment*

On many occasions it becomes difficult for the Veterinarian to find the exact causative factor. Under such circumstances a number of Veterinarians do enucleate corpus luteum with slender hopes that the induced heat may bring about regeneration of the endometrium. This is not correct and if done repeatedly it may damage the ovary. It should always be remembered that infertility is just a symptom which is common to a number of diseases. Attempts should therefore be made to eliminate the causative factors.

### 7. *Cystic Corpus Luteum*

It is difficult to diagnose this condition and it is much more difficult to enucleate the cystic corpora lutea.

### 8. *The treatment of metritis and endometritis*

In practice, veterinarians do resort to enucleation of corpus luteum as a measure in treating cases of metritis and endometritis. In cases of acute metritis, it is not at all advisable to enucleate since the condition may flare up. In chronic cases of endometritis prolonged stimulus to the cervix and uterus is necessary for evacuation of the contents and for regeneration of the endometrium. Naturally occurring oestrogens liberated during induced oestrus due to enucleation of corpus luteum are not very effective being available for a very short duration. It is therefore advisable to use repeated doses of synthetic oestrogens concurrent to the administration of antibiotics or chemotherapeutic agents rather than to depend on a transient heat induced by enucleation of corpus luteum. It is necessary that the veterinarians should refrain themselves from such an irrational measure.

## INDICATIONS

### 1. *To induce abortions in heifers and cows served by undesirable bulls*

In natural breeding many a times such accidents are likely to happen and favourable results have been observed in females which are induced to heat by enucleation of corpus luteum rather than by synthetic oestrogen for prolonged periods.

### 2. *To induce heat in a group of cows at one and the same time and breed them by artificial insemination*

from the semen of high quality progeny tested bull under controlled breeding conditions.

In certain countries of the world like New Zealand, breeding activities are restricted to only certain months of the year. In order to breed a maximum number, in a short span of a week or two in a herd, the Veterinarians import semen from valuable bulls and resort to enucleation of corpus luteum on a mass scale and inseminate them at induced heats. This way the calvings occur in flush season when there is plenty to eat. It is difficult to say as to the degree of the success but the New Zealand Veterinarians claim that the results are fairly satisfactory.

### Technique for enucleation of corpus luteum

The Veterinarian should prepare himself first by clipping nails properly and as far as possible by using armed sleeves and protective clothing. Preferably thin hand glove should be used in order to guard against possible injuries to the rectum that are likely to occur during pressure induced on the mucus membrane while dislodging the corpus luteum from its base.

Before enucleation the Veterinarian should obtain all possible history particularly dates of previous services and actually examine the animals to confirm that there is no early pregnancy and gestational oestrus. The following precautions would be necessary.

- (i) Correct history with regard to calvings, date of last calving, last seen in oestrus.
- (ii) Actual examination to confirm that the animal is not pregnant.
- (iii) Condition of the uterus.

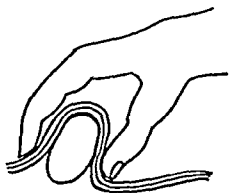
(iv) Whether there is presence of functional corpus luteum? Can it be enucleated with ease?

(v) To ascertain if the cow or buffalo is at the prime of her lactation and is very heavy yielder.

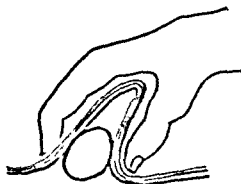
### Procedure

There are two ways of enucleating the corpus luteum in cow and the buffalo, (1) by applying uniform pressure with the finger tips by holding the ovary (2) by enucleating the corpus luteum with the thumb or the index finger by applying pressure at the base. Enucleation of corpus luteum should not be done after heavy feeding or wa-

## REMOVAL OF CORPUS LUTEUM



NOT SO



RIGHT METHOD

Fig. 12. Enucleation of corpus luteum

tering or when the animals are about to be milked. The animal should be properly secured in crate or tied in stable. It should be done in standing position. The Veterinarian should properly lubricate his gloved hand with soft soap and water. The glove should fit in very well and end portions of the fingers should not be loose. The lubricated hand should then be passed into the rectum and on ascertaining the condition of the uterus and confirmation of diagnosis, he should palpate both ovaries very carefully and locate the corpus luteum. Thereafter the ovary should be properly grasped in the fold of the rectal wall between the fingers and the first knuckle of the thumb and thereby causing pressure at the distal end or base of the corpus luteum. If it is a functional corpus luteum it will easily be dislodged from the base and fall into the peritoneal cavity. The tip of the index finger should be kept in the crater for about 20-30 seconds to check haemorrhage. If it is rather hard and in regressing stage, undue pressure shall have to be exerted. For easy manipulation, it is absolutely essential that the rectal fold in which the ovary is caught should be as loose as possible. If the rectal wall is tense, it will be difficult to induce proper pressure and it may also cause damage to the rectal wall. When one is certain that the corpus luteum has been dislodged from the ovary, care should be taken to dislodge it in the peritoneal cavity. If very undue and hard pressure is necessary it is better not to enucleate for fear of damage to the rectal wall and the ovary.

Possible complications as a result of enucleation of corpus luteum

(a) Rectal injuries

Injury to the rectal mucosa and wall are likely to occur if undue pressure is

exerted on the rectum during enucleation of corpus luteum. Injuries may lead to haemorrhage in varying degrees.

## (ii) *Abortions*

In the absence of proper history and when cows are running on pasture free with a bull, it is likely that early pregnancies as that of 4-5 weeks may be missed and in such cases, if enucleation is done it may lead to abortion. A number of instances are on record under Indian conditions that such an enucleation from 4-5 weeks has led to abortions in cows and buffaloes.

## (iii) *Adhesions*

Adhesions frequently follow the rough handling of ovaries during enucleation. If the enucleated corpus luteum is dropped in the ovarian bursa, it may cause severe adhesions with likelihood of closure of the fimbriated extremity (ring adhesion) of the fallopian tube leading to permanent sterility. However, this is dependent on the degree of adhesions. Adhesions may remain only as bursal and occlude the fallopian tubes.

### (a) *Adhesions between ovary to bursa*

This may occur in a number of cases

### (b) *Adhesions of the bursa and occlusion of the tubes*

The enucleated corpus luteum may drop in the ovarian bursa and may remain there. Blood coagulation may form inside the bursa and the surface of the enucleated ovary may get itself attached to the bursa and cause occlusion of the tubes. If there is any infection at the same time either ascending or descending from the uterus or abdominal cavity, it may lead to salpingitis. Springs (1945) examined 1250 infertile cows and heifers and found fifty cases of adhesions. Moberg (1954) reported

that the pathological changes in fallopian tubes and ovarian bursa may occur as a result of complications of removal of corpus luteum leading to bursal adhesions. Ovarian adhesions caused due to rough manipulation of the ovary during enucleation of corpus luteum or massage treatment is a common cause of permanent sterility. Deshpande (1963) observed the incidence of bursal adhesions as 12.5% in the slaughter house material of Murrah buffalo genitalia.

## (iv) *Bleeding from the ovary*

This occurs in almost all the cases after removal of corpus luteum. The amount of blood may be anything from few ounces to a pound or even more which accumulates slowly into the peritoneal cavity. In some cases haemorrhage from the ovary may continue due to defective coagulation of the blood. Animal getting fed with a very high oxalate content which leads to depression of calcium level in the blood with consequent tendency to haemorrhage and defective formation of coagula. In Sweden, during autumn the cattle are fed on sugar-beet leaves which contain a high percentage of oxalate and the Veterinarians in that part have to be very careful in enquiring the history and not to adopt enucleation in such cows fed on feeds rich in oxalate content. In India, the paddy straw contains high level of oxalates and as such care also should be taken to avoid removing corpus luteum from cows and buffaloes in areas where paddy straw is the main component of the cattle feed. Teige (1955) considered that fatal haemorrhage on enucleation of corpus luteum can occur only in the absence of normal levels of prothrombin content in the blood. The amount of the bleeding is dependent on the type of corpus luteum enucleated.

Hackhansen (1950) divided this condition in five groups. (i) Bleeding due to removal of functional corpus luteum. (ii) Bleeding due to removal of persistent corpus luteum. (iii) Bleeding due to removal of pregnancy corpus luteum. (iv) Bleeding due to removal of corpus luteum in pyometra. (v) Bleeding due to removal of cyst.

In his first experiment there were twenty-five cows between three and eleven years with functional corpora lutea for enucleation. Some cows showed rise of temperature, high pulse, restlessness and exhaustion after removal of corpus luteum. These cows were slaughtered between nine to thirty-two hours after enucleation. In three cows there was one litre of blood; in seven cows there were two litres and in the remaining fifteen cows three litres of blood.

In the second group there were thirty-two cows between three to twelve years of age having persistent corpus luteum. They were slaughtered between two to thirty-six hours after removal of corpus luteum. In two cows the quantity of blood was one litre, and in the rest from a few ml. to half a litre.

In the third group there were four cows between three to nine years of age with pregnancy corpus luteum. On enucleation, there was rise of pulse, accelerated respiration, restlessness and exhaustion. In one case there were symptoms of abortion. They were slaughtered between twenty to thirty hours after removal of corpus luteum. The bleeding in one case was four litres, in two six litres and in one seven litres.

In the fourth group, there were thirteen cows between three to nine years

of age having pyometra. On enucleation of corpus luteum, there was rise of pulse, accelerated respiration, restlessness and exhaustion. In nine cows restlessness began after seven hours. On enucleation, they were slaughtered between eighteen to twenty-eight hours. In all the cows, bleeding was to the extent of about four litres.

It can be gathered from the above experimental work that bleeding invariably occurs from the ovary on enucleation of corpus luteum. The degree however will differ depending on the type of corpus luteum enucleated.

In most cases bleeding will be very trivial and stops very soon due to coagulation. The first symptom of profuse bleeding is restlessness, and tympanitis, which may occur in an hour or so after enucleation. This may cause irritation of the peritoneum, which may interfere with normal ruminal movements resulting in tympany. In most cases tympany will disappear in about an hour or two without any complications. Fatal bleeding may also occur. Teige (1955) reported the incidence of fatal haemorrhage as 1:1000. Stener (1955) described ovarian colic after removal of corpus luteum, marked restlessness, striking the abdomen with hind feet, lying on one side and again standing up, arched back, raising of tail, suspended rumination and fall in milk yield. He mentions that these symptoms may pass off in an hour or so but in some cases may last even for a day. The colicky symptoms are associated with tympany and internal bleeding which may end fatally.

Severe bleeding may be indicated by sudden weakness, shivering, pale mucous membranes, weak pulse, tympany, colicky pains, anxious look and tendency to lie prostrate. Goetze (1955) found a

correlation of fatal cases with the state of nutrition level. The chances are more amongst the heavy milkeis.

Sohoni (1967) reported on his experience at the Government Milk Colony, Aarey, that there was no fatal bleeding or symptoms of tympany on the removal of corpus luteum in buffaloes. Sane (1953) reported two cases of fatal haemorrhage in heavy milking Murrah buffaloes as a result of enucleation of corpus luteum. Marked tympanitis, restlessness, pale mucous membrane and cold extremities were the predominant symptoms.

### Treatment in cases of excessive bleeding

(a) Injections of normal saline and calcium borogluconate may be given.

(b) Vit K and coagulum may also be tried.

(c) In threatened conditions, ligation to the ovarian blood vessels may be done by surgical intervention per vagina.

(d) Blood transfusion up to about five litres may be given.

(v) *Fibrosis of the ovary*

Fibrosis may result due to undue compression of the ovary in trying to dislodge hard corpus luteum. If the enucleation is done too often, it may lead to fibrosis of the ovary. If enucleation of corpus luteum is done, the particular ovary should not be subjected to repeat removal.

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## Chapter 19

# Ovulation, Fertilization, Implantation, Gestation, Placenta and Placentation

### OVULATION — FERTILIZATION

Fertilization is the union of a viable ovum and spermatozoan. After ovulation the discharged mature ovum is carried by the sweeping action of the fimbriated extremity of the fallopian tube and if the mating has occurred in good time, the spermatozoa are present at the site of fertilization to fertilize the viable ovum. In most of the mammals the union of ovum and sperm usually occurs in the pavilion of oviduct. The ovum after its release from the Graafian follicle is surrounded by granulosa cells — corona radiata. Disintegration of these cells occurs due to the hyaluronidase present in the galea capitis of spermatozoa (Blom & Anderson, 1965) which dissolves the intercellular contents that makes penetration of the sperm easier. Normally only one sperm is necessary for fertilization, but without a total optimum concentration the conception rate declines. Observations show that, if insemination is done by a sample of semen containing less than 10 million sperm/ml, conception rates drop even though theoretically only one sperm is necessary for fertilization. This evidently proves that there is a cumulative effect of the sperm concentration at

the site of fertilization. Walton (1933) demonstrated that basically fertilization was dependent on the number of spermatozoa deposited in the female tract. All the spermatozoa do not reach the fallopian tube but the greater the number deposited on ejaculation or insemination, larger number reach the site of fertilization. Whatever be the number of sperms deposited in the vagina after ejaculation, till they reach the pavilion of the oviduct, a large number are held up at several places in genital tract such as vagina, cervix, body of the uterus, both cornua and fallopian tubes with a progressive loss during transit. Cheng and Casida (1948) reported failure in conception in rabbits inseminated with less than 20,000-100,000 spermatozoa per insemination. Willet and Larsen (1952) reported a decline of 0.6% conception rate in cows with decline of each million from 120 million to 40 million. Rapid decline in conception rate of 3.6% occurred below this level.

Random movements of the sperm bring them in contact with the ovum and they congregate around it. It has not been proved if this is due to the real chemo-taxis. But the secretions of ova in certain species serve to trap the



sperm. The sperm swim in a spiral path and when they come across a solid body like that of ovum, they move towards it. A positive tactile response (thigmotaxis) keeps the sperm head attached to the ovum. After dissolving the cells of the corona radiata the spermatozoa attach to the zona pellucida. If the spermatozoa come in an oblique direction towards the egg, the contact flattens out the spiral which keeps the sperm head without penetration, but, if the sperm approaches the ovum in radial direction penetration does occur. Only motile spermatozoa are capable of making the attachment, then the sperm penetrates the zona pellucida and apparently comes to rest against this membrane. On the surface of egg, a cone or a long filament of cytoplasm is formed with which a sperm head comes in contact. The cone then retracts and draws the sperm into the egg engulfing it. Sometimes more than one sperm may get entry into perivitelline space, and this condition is known as polyspermy. However the development is then abnormal and soon ends. Usually the tail is shed off and the sperm head (male pronucleus) then rotates and advances towards the female pronucleus. The union between the male and female pronuclei results into the formation of a zygote with its full complements of chromosomes. It takes nearly eleven hours for complete process of fertilization in rabbits (Austin and Bishop, 1957).

The spermatozoa loses its tail after penetration in the ovum and ovum becomes impervious to any other sperm following the union; the sperm head which becomes the true pronucleus unites with female pronucleus. On formation of the zygote actual cell division commences. In most animals it takes 3-5 days for the fertilised ovum to reach the

uterus. By this time the zygote develops to many celled (96), morula stage of segmentation.

The site of semen deposition varies in different species. In the cow and ewe semen is deposited in anterior vagina and cervix after ejaculation. In the mare, sow and bitch semen is mostly deposited within the cervix and uterus. In sow and bitch vagino-uterine waves during coitus facilitate intrauterine deposition of semen after ejaculation.

In the transportation of sperm, muscular contractions of the vagina, cervix and uterus are primarily important. The waves of contraction are antiperistaltic. Other factors such as chemotaxis etc. are also accountable. Millar (1952) demonstrated that in consequence of female orgasm, negative pressure develops in the uterus which facilitates rapid passage of semen through the uterus. When the sperms reach the apex of the uterine cornua, further transport in the fallopian tubes is dependent on the motile action of cilia and the segmental contractions of the fallopian tubes.

Sperm can live for a limited time in the vaginal passage and they do not survive for more than 6 to 12 hours. The cervix is considered to be an ideal site for survival of the spermatozoa. This is favoured due to characteristic mucus secreted during oestrus. Sperm survival is very poor in the genital tract of the female which is not in heat. In most of the animals the viable spermatozoa will survive in the cervix for about 36-48 hours or even longer. Maximum concentration of the sperm is usually found in the cervix as compared to uterus. Anderson (1922) reported that the stallion spermatozoa can live less than 6 hours in the vagina and for about 12 hours in the uterus. Sperm transport in

the female genitalia is very rapid Van Demark and Moeller (1951) reported that in the cow, the sperm to reach the fallopian tube takes 25 minutes after insemination Rao *et al* (1960) reported in buffaloes that it takes only 4 minutes for the spermatozoa to reach the fallopian tube after insemination Stark (1949) reported that in the ewes, it takes 6 minutes after natural mating Van Demark and Hays (1952) reported that non motile and motile spermatozoa take the same time and the transport is rapid to the site of fertilisation

The life of the spermatozoa and ovum is of a short duration and as such chances of fertilization are greatly dependent on the time relationship between ovulation and insemination or natural service In domestic species such as mare, sow and bitch, in which oestrus continues after ovulation, late service or insemination may become futile owing to the short life of the ovum In the cow, since ovulation occurs few hours after the end of oestrus, the effects of late insemination are not very marked when inseminations are done within a reasonable time at the end of oestrus or within a few hours after the cessation of oestrus Barrett (1948) showed that cows inseminated 2 to 4 hours after ovulation, had a conception rate of 75% and all the embryos were normal at 35 days In cows which were inseminated 6 to 12 hours after ovulation the conception rate was 60 to 75% but only 30% of the embryos were normal at 35 days, which shows that there is perceptible decrease during nidation and further progressive development Cows which were inseminated 12 hours after ovulation resulted in low conception rates and with hardly any normal embryos It can thus be gathered that in cows the unfertilized ovum can remain viable for 2 to 8 hours only and thereafter the viability is

rapidly on the decline and degenerative changes begin In case the ovum is not fertilized it fails to divide normally and degenerative changes occur, as it passes down through the fallopian tubes The unfertilized ovum is fragmented in cytoplasmic segments of unequal size and this fragmented ovum many a times may resemble a fertilized ovum The unfertilized ova completely disappear through the uterus by disintegration or phagocytosis

## IMPLANTATION

The fertilized ovum descends down the fallopian tubes and it has a loose shell while it enters the uterus The early cleavage of the zygote is completed in the fallopian tube and the young embryo consisting of 8 to 16 cells or in the blastocyst stage enters the uterus for permanent attachment There is no abundant yolk in the mammalian egg which supplies energy for the growth of young embryos It is therefore necessary for the mammalian young to draw its nutrients from the uterus proper

Implantation of the embryo can be said to have occurred when it gets a firm hold in the uterine lumen and thereafter it does not float freely in the uterus On arrival in the uterus, the embryo is dependent on the uterine secretions for obtaining necessary energy The glands of the uterus secrete 'uterine milk' composed of proteins, fat and traces of glycogen The young embryo derives its sustenance from the uterine milk and the cellular debris from the epithelial lining of the uterus until its stage of implantation and until the formation of permanent placental connections with the maternal circulatory system

The fertilized ovum arrives in the uterus at a stage when proliferation of

the endometrium has occurred under the influence of the progesterone. On arrival, the fertilised ovum lies free within the lumen of the uterus and it continues to divide there. The cellular division further continues and a cavity filled with fluid, develops in the centre of the developing zygote, when it becomes a blastocyst. During this stage, it has an outer spherical layer of flattened cells the trophoblast and a clump of cells attached internally to form the inner cell mass. At about this time, the zona pellucida disappears and the trophoblastic cells get attached to the endometrium.

The fertilised ovum is partly dependent on its own cytoplasmic stores and partly on the secretion of the endometrium called uterine milk for its growth and division.

At this stage the placenta begins to develop which serves to provide nutrition to the foetus as organ of foetal respiration and excretion. These functions are achieved by the close approximation of the placenta with the endometrial capillaries which keep in direct continuity with the foetal circulation. The area involved and the intimacy of contact varies from species to species. There is an interval during the period between the formation of blastocyst and full development of the placenta. During this period, foetal membranes will be found to lie free within the uterine lumen when there is no close connection with the uterine wall.

### *Hormonal effect*

In the process of implantation progesterone is actively concerned in determining the preimplantational changes in the uterus. The balance between progesterone and oestrogen is more important than the level of progesterone

alone. During the progestational period the muscular activity and tone of the uterus is considerably decreased which helps to retain the blastocyst in the uterine lumen. Increased vascularity also develops to provide rich blood supply to the uterine epithelium. In addition to this nutritive material chiefly composed of glycogen and fat accumulates in the uterine epithelium. This combined with cellular debris and extravasated leucocytes in the lumen of the uterus forms the histotrophe i.e. the uterine milk. This provides nourishment to the embryo in the early period of its uterine life before the chorio-allantoic placenta is established.

In the rabbit from 80 hours after coitus, the histotrophe plays a vital role. In the sheep it is from about the 9th day.

### **Course of implantation in farm animals**

In farm mammals the course of implantation is a gradual process. The attachment of the trophoblast to the endometrium during the early period is very loose.

### **Cow**

In about a week after fertilisation the zonapellucida is shed in the early blastocyst stage. In a few days thereafter the blastocyst starts elongating. The gastrulation is completed at 13 days. By about the 33 days, the chorion forms a fragile attachment with 2 to 4 cotyledons surrounding the foetus. In a few days thereafter the maternal and foetal tissues become intimately inter-digitated and the embryo is nourished by these cotyledons. The progressive development of the cotyledons is presumably due to progesterone.

## Mare

In the mare it takes about two months for the implantation to begin even though the blastocyst is quite close to the uterine epithelium. By this time it reaches the diameter of about 5 cm. The blastocyst acquires an albumin coat during the third week of fertilisation preceded by a group of columnar cells, the trophoblastic disc, which can be seen in the trophoblastic wall of the chorion. The discs are concerned with the ingestion of uterine milk, though their function is to help the attachment. By about the 10th week the villi from the chorion penetrate into mucosal folds of the uterine wall and by about the 14th week implantation is completed.

## Ewe

The development of the blastocyst in the ewe during early stage is similar to that in the cow. The blastocyst attachment may occur even as early as the 10th day and as late as 17th day after fertilization. By about the third week the blastocyst may attain a length of 4 cm. (Chang and Rowson, 1965). The chorion expands and fills the uterine lumen by about the 18th day which brings the trophoblast in close contact with the uterine epithelium on the caruncles. Eventually the trophoblastic cells invade and destroy the uterine epithelium wherever they are in close proximity. This brings the chorion in intimate relationship with the maternal tissue. In the ewe the process of implantation is completed by about 4th to 5th week.

## Sow

The development of the blastocyst is more or less similar to that in the ewe. In the sow 12 to 24 days period is required for attachment after fertiliza-

tion. In about a week's time, the zona-pellucida surrounding the blastocyst is shed with the result that the trophoblastic cells are brought in direct contact with the uterine epithelium. From now onwards there is rapid proliferation of the trophoblast. The endoderm appears and the blastocyst changes from a small spherical vesicle to an elongated thread like tube which attains a length of several centimeters. Throughout this early period of attachment, the embryo draws its nutrition by absorption of the histotrophic or uterine milk. The blastocyst in the sow provides a long length which enables a very large absorptive surface.

## Duration of pregnancy and factors involved

The gestation period varies in different species. However considerable variations are also observed within species. A variety of factors are responsible for causing variations in the duration of gestation. It is usually observed that larger the size of species the longer is the gestation period. Differences between breeds are also observed. It is more marked in mares as compared to cows, ewes and sows. Within breeds the small variations may be due to the mating time in the breeding season, influence of the season, management and feeding practices and also individual variations from pregnancy to pregnancy.

In general, gestation may be defined as the period which extends from fertilization of the ovum to the expulsion of a fully developed and viable calf. In the mare and the bitch duration of oestrus is longer with uncertainty of ovulation time as such the exact parturition time can not be predicted. Wide variations observed in the mare can be attributed to delayed implantation. The

duration of gestation is closely related to the breeding season and is markedly observed in seasonal breeders, which is evident by the major number of births that occur at such a time of the year when food is plentiful.

Variations within species have also been observed which are related to the litter size. The larger the size of the litter the shorter the gestation period.

### Hereditary Factors

The duration of pregnancy is influenced by hereditary factors, Rollins *et al* (1956) reported that the genotype of the foetus inherited from both the sire and the dam has effect on the gestation period. Rollins and Howell (1951) also observed in Arabian horses that the sex-linked genes in dams and foetuses have an influence on the gestation period. Terrill & Hazel (1947) reported that the difference in gestation period between mutton breeds of sheep with different wool types are attributable to genetic factors. Similarly it has also been observed in beef cattle and goats that the genetic constitution of the foetus plays a vital part in the length of gestation period.

It may be cited here that the Holstein Friesian cattle have a mean gestation period of 279 days, whereas it is 290 days in the Brown Swiss.

In the hybrids, maternal influence is markedly observed. This is evident from the fact that the mare has gestation period of 335 days while as in the ass it is 365 days. The mare carries a hybrid for 345 days, which is obviously less than the mean between the species, but nearer to her own period of gestation, while as the ass carries a hybrid of 355 days, which is more than the mean average and nearer to her own period of gestation.

It can thus be gathered that the length of gestation is controlled by the hereditary influence and the genotype of the dam and foetus. The other possible factors may be accounted for to the endocrine mechanism, size of the foetus and foetal metabolism.

### Season

Chaudhari and Sinha (1951) reported in Tharparkar cows that there is a tendency for the gestation period to be lower during the period between the end of winter and beginning of summer and also between the end of rainy season and beginning of winter i.e. when the climatic conditions are fair. The length of gestation period in male and female calves tends to be longer when calving is due in rainy season. Brakel *et al* (1952) reported that cows freshening in winter have a longer period of gestation than those in summer. They also observed differences in the duration of pregnancy between spring i.e. March to May and Autumn i.e. September, October and November calvers. For calves born in the spring, the duration of pregnancy was on an average 2.07 days longer than those born in autumn. Kohli and Suri (1957) reported in Haryana cattle that the longest gestation period both for male and female foetuses was of  $297.1 \pm 3.2$  days and  $293 \pm 1.8$  days during October calvings. The overall average reported was  $290.73 \pm 0.91$  days. Singh *et al* (1958) reported in Tharparkar cows, that there was no significant effect of the year and season of calving on the length of gestation period of both sexes. Clegg (1959) summarised that the effects of breeding season on the length of gestation period are primarily associated with the available nutrients in the environment, to which the animal is exposed during the period of gesta-

tion Rao (1966) reported in Malvi cattle that the winter born calves were carried for 282.69 spring calves for 283.35 summer calves for 285.11 days and calves during fall season were carried for 285.25 days. Hadi (1966) reported in Deoni breed that male calves born during November to February (winter months) were carried for 285.76 days and females for 284.43 days. For calvings that occurred from March to June (summer), the male calves were carried for 281.97 days and the females 283.71 days. For calvings occurring from July to October (rainy season) he reported that the male calves were carried to 281.76 days and the female 285.15 days. In Gir cows Dunge (1969) observed that gestation period was shortest (283.22 days) during winter and longest (283.37 days) during rainy season. Longer gestation periods — 285.21 days for male and 283.00 days in female were observed in spring and summer respectively where as shorter gestation periods — 282.13 days for male and 282.23 days for female were observed during summer and rainy seasons respectively. These differences on account of seasons were non significant. Rajulu and Rao (1966) reported observations on Ongole cows that the male calves born in February, June and December were carried for a longer period than those of March, April and October. Female calves were carried for a longer period in January, July and August and shortest in February, March and September. For both sexes combined the gestation period was longest in June and December and lowest for March, May, September and October. The differences were significant.

Ragab and Asker (1951) reported that in Egyptian buffaloes the month of calving had a significant effect on the length of gestation period. Arunachalam

*et al* (1952) reported in Murrah buffaloes that the month of calving had no effect on the length of gestation periods. Similar observations were made by Nambiar and Raja (1962) in Murrah buffalo cows in Kerala. El Dessouky and Rakha (1964) reported that in Egyptian buffaloes the gestation period averaged  $317.98 \pm 1.82$  days and was not significantly affected by seasons and month of calving. El Sheikh (1967) reported that the gestation period in Egyptian buffaloes was significantly shorter during the period from May to November compared to the rest of the year. He also mentioned that during winter and spring the buffaloes are better fed since it is the time of the year when plenty of clover is available. The climate during winter and spring months is said to be mild.

In the mare delayed implantation is considered as the major factor for the seasonal differences in the length of gestation periods. Foals born in summer have a shorter gestation period while as the winter foals have the longest. Mares that are bred in late spring after due conditioning may stimulate a rapid development of the foetus to start with. It is not clearly known if implantation is influenced by seasonal factors in mare. Howel and Rollins (1951) reported that in the mare in contrast to cows the breeding season has a marked effect on the variation of gestation length. The influence is to the extent of 11% of the total variance. Arabian mares bred from December to May had a gestation period of 10.1 days longer as compared to those bred from June to November. Dusec (1967) reported (on a data obtained from 1119 Klandrats or English half bred mares) the maximum average gestation period for mares foaling in June as 340 days as compared to the minimum of 328 days for those foaling in September.

Asdell (1929) reported that the month of conception influenced the length of gestation in the goats. The average length for conception in August was  $151.3 \pm 0.1$  days whereas during February it was  $149.8 \pm 0.1$  days. These differences are attributed to the nutritional effects and may be due to insufficiency of feeds or because of more energy required to maintain body temperature during winter. Terrill and Hazel (1947) reported that in ewes longer gestation period has been recorded in those bred early during the breeding season. The average decrease of 0.03 days in the duration of pregnancy for each day during the breeding season was highly significant. Singhvi (1964) reported that lambs born in January-February were carried longer as compared to lambs dropped in August-September. Gupta *et al* (1964) reported that Black Bengal Goats had a mean gestation period of  $144.3 \pm 0.17$  days. Seasons-wise gestation periods were as follows: winter — 145; summer 144.12 and monsoon — 144.07 days. Significant differences were observed between summer and monsoon gestation lengths.

Bhattacharya *et al* (1967) reported in large white pigs that the gestation periods for summer farrowings were longer than those for monsoon and winter but the differences were nonsignificant.

### Nutrition and Pregnancy

Knowledge with regard to the nutrition of the foetus during various stages of gestation is not fully known. A considerable number of fertilized ova do perish during early stages of gestation, resulting in low fertility. In all species of the farm animals there is fair amount of mortality in the newborn. Hammond (1963) observed in the pigs and sheep

a fairly high mortality to the extent of 20 to 25 per cent in the newborn, particularly when weather conditions are bad at lambing. He also observed that mortality is highest amongst the smaller ones of the lot at birth. From nutritional point of view Hammond (1963) mentioned the following distinct stages during pregnancy.

*The blastocyst stage:* When the developing embryo lies free in the uterine cavity, nutrition is supplied from uterine milk secreted from the glands of the uterus. This provision lasts for about three weeks.

A fairly good number of fertilized ova perish during the blastocyst stage. This is the main reason as a result of which even in the most fertile bulls and best insemination technique, conception rate cannot go higher than 75% even with the best managed herd. In most of the cases there is no suspicion of pathological condition and the usual assumption is that the death of fertilized ova is due to lack of sufficient nutrients for the growth. There is experimental evidence in rabbits which indicates that fatty condition of the dam increases the percentage of foetal death in the blastocyst stage.

During this stage from about the 20th day until 6 to 8 weeks before birth, the outer cells of the foetal membranes, the trophoblast cells erode and grow into the maternal tissue. They are like cancer cells which have priority of nutrition over practically all the tissues of mother's body. Thus whether the dam is gaining or loosing weight during this stage, the foetus gets all the required nutrition. On account of such a provision in the ewes, it is commonly observed that at the 90th day of pregnancy the foetus is of the same weight irrespective

of whether the ewe has been well or poorly fed or whether she carries singles, twins or triplets. During the embryonic stage, the placenta is growing in the area because the trophoblastic cells are still active. The area over which the trophoblastic cells extend is important for the nutrition of the young one in the subsequent foetal stage after the trophoblastic cells have perished. During that stage the area of the placenta will limit the nutrients the young will receive, affecting the weight at birth. A number of factors limit the area that the placenta occupies. The large number of embryos present will decrease the area for each. Thus twin lambs have on an average about 55 placental cotyledons each as compared with 80 for singles and these weigh on an average 365 gm each as compared with 532 gm for singles at the end of pregnancy.

In multiparous animals such as dogs, pigs and rabbits, when litter size is large, few are of smaller size. This is attributed to the limited space within the uterus. It is difficult to rear the under-sized ones. Size of the uterus also limits nutrition in the foetal stage on account of the limits on the area of the placenta. This can be proved when reciprocal crosses are made e.g. crossing small Shetland pony and the large Shire horse. In the small dam, the cross bred has been limited in growth by the area of the placenta whereas in the large dam it is only limited by the genetic constitution of the sire. Therefore the ultimate adult size is determined during foetal life. This is well shown in the difference in size between mules and Hinnies. In the reciprocal crosses between small and large breeds in cattle and sheep, birth weight is affected as in the horse but the calf and the lamb do not develop to full development at birth as in the horse. The reciprocal

crosses therefore tend to become closer together in size in later life.

Crosses between the Charollais bull and the small Jersey cow in Denmark show no increase in calving difficulties. Large amounts of fat in the abdomen at midterm prevents full expansion of the uterus with the result that it limits placental area and so the birth weight.

*The foetal stage:* In foetal stage the trophoblast cells die and nutrition is assisted by transfusion from maternal to foetal blood streams. This period occupies the last 6 to 8 weeks of pregnancy in farm animals. The foetus is absolutely dependent on the available nutrition from the dam. It is therefore evident that feeding of the dam is extremely important during the foetal stage. Low plane of nutrition in the dam has a greater effect in decreasing the weight of the twins than of a single lamb, while as on the high plane of nutrition the weight of the individual twins gain normal weights. During the foetal stage, there is flow of sugar from the maternal blood to the liver and muscles of the foetus where it is stored as glycogen. Immediately after parturition this acts as a source of energy for young one, enables to maintain body temperature and gives muscular strength for suckling.

If the dam is poorly fed during the foetal stage, the glycogen store is very small. Likewise the storage of iron during the late stage of gestation for the prevention of anaemia during the suckling stage occurs in the same manner as that of glycogen (Hammond, Jr. 1963).

#### Gestation period

Kohli and Suri (1957) reported a significant coefficient of correlation of 0.14% between body weight of the dam



and gestation period, i.e. heavier the cow the longer the duration of gestation and vice versa.

Howell and Rollins (1951) observed that a pregnant mare on a better plane of nutrition has a gestation period shorter by about 4 days as compared to those reared on pasture and oat hay.

Thomson and Thomson (1919) reported that the mean gestation period in ewes on low plane of nutrition during the second half of gestation was somewhat reduced in case of twins, however in ewes bearing single lamb there was no influence of the plane of nutrition. Alexander (1956) observed in Merino ewes placed on high, medium and low planes of nutrition from the 108th, 129th, 136th or 143rd day of gestation to parturition, that the period of gestation in ewes on sub-maintenance ration was reduced by 0.7 to 5.0 days. Gestation period was advanced in those with heavier uterine contents at the time when severe under nutritional treatment was commenced. His observations show that the extent of reduction of gestation length as a result of under nutrition was greater in advanced foetuses than in the younger or in twins than in singles.

Gupta *et al* (1964) reported the influence of sire on gestation in Black Bengal Goats and recorded significant differences in gestational lengths between a number of sires from 142.23 to 145.25 days. Arunachalam *et al* (1952) reported that in Murrah buffaloes the sire of the foetus had no influence on the duration of gestation period.

Dange (1969) observed influence of sire on the length of gestation period in Gir cows. Irrespective of sex of the calf the gestation period varied from maximum period of 287.50 days (bull

Manohar) to the minimum of 281.22 days (bull Manoj). The differences in gestation period on account of sire of the calf were significant. He also further estimated heritability of the gestation period as a function of the genotype of calf. The heritability value estimated by intrasire daughter-dam regression was 0.26 and intrasire daughter correlation was 0.22.

El Shaikh (1967) reported that the Egyptian buffaloes have a longer gestation period than the Indian or Pakistani buffaloes which he attributed to breed characteristics. The average gestation period of 1746 buffalo calvings was  $316.68 \pm 0.19$  days (S.D. 8.21 days and C.V. 2.56%). The modal length was 316 to 320 days.

Bhattacharya *et al* (1967) reported in large white pigs that the influence of boar on gestation length was insignificant. The means reported for different boars varied from 114.83 to 116.00 days. Singh *et al* (1958) reported in Thaparkar cattle that the existing small variation in the gestation periods in that herd was of very little genetic influence.

Dominiques (1961) reported that Brazil buffaloes have rather short gestation periods extending from 270 to 280 days. This is in contrast to the average of 310 days in Indian buffaloes.

#### Gestation Period in Sheep and Goats

Roy *et al* (1962) reported in Bikaneri Sheep gestation period, as  $150.1 \pm 0.29$  days.

Roy *et al* (1962) reported in Jamnapari goats a gestation period of  $146.5 \pm 1.2$  days.

Sudersanan and Raja (1973) reported in Malabari goats the mean gestation period of single, twin and triplets preg-

nancies as 145.2, 147.2 and 146.3 days respectively. The incidence of single, twin and triplets in goats was 47.0, 35.29 and 17.65% respectively.

Oane (1936) reported that in the highly bred cats which have little exercise, the gestation period extends to 65 days as compared to the average of 63 days as reported by Gross (1936).

Ineichen (1948) reported that the length of gestation period in heavier cows is higher as compared to that of lighter ones.

Bhattacharya *et al* (1967) reported in large white pigs that the age of sow at farrowing had no significant effect on gestation period though the gilts had slightly longer gestation periods than the sows.

Alexander (1956) observed that the mean duration of gestation was decreased by changing ewes to low level of feeding in late pregnancy.

Reid and Hiwks (1962) observed that ewes fed *ad lib* had shorter gestations than those on constant or rising intake.

### Internal Environmental Factors Affecting the Length of Gestation Period

The internal environment is related to the status of physiological condition of the mother i.e. age, weight, number of pregnancies and inherent potentiality for effective reproduction. In addition to the endocrine constitution of the dam which influences the internal environment there are other factors such as the size and sex of the foetus and size of the litter specific to the species.

### Number and size of the foetuses

Knapp *et al* (1940), Braude and Walker (1949), Brickel *et al* (1952), and Rollins *et al* (1956) reported that

in dairy and beef animals there is positive correlation between the size of the foetus and length of gestation. However in case, the intrauterine life is prolonged, the increased weight of the calf is merely the result of continued growth gained after the due period of gestation. In ewes the size and the weight of foetuses have similar effect on the duration of pregnancy as in cows. In sows no such effect has been observed. In the cattle, twins are carried 3 to 6 days less as compared to the average gestation period of a single foetus. Alexander (1956), Bonadonna and Valerani (1946), reported in sheep that the twins are carried 0.6 days less than the single births. Asdell (1929) and Hinterthur (1933) reported that in goats, though the differences in the duration of gestation are not significantly associated with the number born, the multiples may be carried for a shorter term. Joubert and Bonsma (1957) and Krizenecky (1935) reported that in pigs the size of the litter does not exert a marked effect on the mean gestation length. It can thus be gathered that in the cow and mare, the twin and multiple pregnancies had shorter duration of gestation period as compared to sheep and goats, in which twin pregnancy is more or less the rule. In sows however the total weight of the foetuses counts towards the length of the gestation period rather than the number of foetuses.

Nambiar and Raja (1962) reported in Mavalai buffaloes in Kerala a highly significant correlation of birth weight and the length of gestation period of  $\pm 0.477$ . Similar findings were also reported by Ragab and Asker (1951) in Egyptian buffaloes.

Iorbes (1967) reported in Scottish half bred ewes the mean gestation period of

146.57 days. Singles were carried significantly longer than twins or triplets.

### **Influence of the Sex of the Foetus on Gestation period**

In the cows and mares there is usually significant influence of the sex of foetus in determining the length of gestation period. Brackel *et al* (1952), Mc Keown and MacMohan (1956) and Rollins *et al* (1956) reported in dairy breeds and Burris and Blunn (1952), Knapp *et al* (1953) in beef breeds that the duration of gestation is longer by one to two days in case of male calves. It is evident from the work of Spencer and Knodt reviewed by Mc Keown and MacMohan (1956) that in contrast to the observations in cows, the gestation period in human was shorter for male than for females. Purbey (1965) recorded in Dangi cows an average gestation period of  $286.0 \pm 1.2$  days for male calves and  $283.5 \pm 0.5$  days for female calves. The male calves were found to be carried for 2.5 days longer than the female calves. Phatak (1965) observed average gestation period in Gir cows as 285.00 days  $\pm$  4.5 days (165 observations), males were carried for 289 days and females for 285 days.

Tatke (1967) reported average gestation period in Khillar cows as 287.06 days (718 observations), further it was observed that male calf was carried for 288.00 days and female calf for 286.25 days. Sane *et al* (1969) stated the gestation period for Gir cows as  $281.00 \pm 19$  days (199 observations) and for Sindhi cows as 287.00 days (348 observations).

Dange (1969) reported overall mean gestation period in Gir cows as  $283.30 \pm 0.14$  days (391 observations) while for male calf it was  $284.20 \pm 0.21$  days and for female calf  $282.38 \pm 0.19$  days.

The difference on account of sex was significant. The overall mean gestation period and the gestation period for male and female calf in the same breed as reported by Ponkshe (1969) was 287.86 days, 288.5 and 287.33 days respectively; (228 observations) but difference due to sexes was non-significant. Sonawane (1969) studied gestation period in Gir and Rathu cows and reported average gestation period as 280.85 days (300 observations) for Gir and 281.19 days (366 observations) for Rathu cows. The gestation period of Gir cow that carried male and female calf was found as 281.52 days and 280.23 days respectively while in Rathu cows the corresponding gestation period was 281.59 days and 280.81 days respectively. The differences in gestation period due to sexes were significant in both breeds.

Hadi (1966) reported that in Arab Thoroughbred Mares at Hingoli (India) that the colts were carried on an average 0.14 days longer than fillies.

Singhvi (1964) reported that in Jaisalmeri sheep of Rajasthan (India), the gestation period of males is more than that for females. Males born for August-September were carried for 152.96 days and that for January-February, 152.23 days. Females born for August-September season were carried to 152.16 days and that for January-February season, 151.76 days. Similar effects have been recorded in crossbred sheep (Jaisalmeri females  $\times$  Marwari rams). Kumaran (1956) reported from a study of 1488 gestation periods of Tharparkar cows at Hissar (India) an average of 290 days for male calves and 288 days for females. Rao (1966) reported in Malwi cattle that the male calves were carried for 283.86 days and the females for 284.18 days. Dave (1911) reported a mean gestation period of 283 days in Red-Sindhi

Howell and Rollins (1951) reported a slight effect of age of mare on the period of gestation.

Sane *et al* (1969) in their observations on a Gir herd which was partly stallfed and partly on pasture observed that the average gestation period extended to  $281 \pm 1.9$  days. The average duration of pregnancy in primipara was  $270 \pm 1.87$  days, that of the secondipara  $276 \pm 1.26$  days and from the third conception onwards the length of gestation period was observed to be constant round about 284 to 285 days. They recorded a gestation period of  $287 \pm 2.83$  days in stallfed Sindhi cows and the duration of gestation was rather variable from calving to calving.

Sane *et al* (1969) recorded an average gestation period of  $311 \pm 0.35$  days in stallfed Surti buffaloes. The length of gestation period was slightly variable and there was no consistency from calving to calving denoting that the age of the dam had apparently little or no effect on the duration of pregnancy.

Bonsma (1939), Joubert and Bonsma (1957) and Asdell (1929) reported their findings on sheep, pig and goat respectively, that the sequence of conception has very little or no influence on the length of gestation period. Terrill and Hazel (1947) recorded in the ewe an average increase of 0.27 days in the mean length of gestation for each advancing year of age.

Pearson and Enders (1911) reported that in pigs the older sows have longer gestation periods than younger ones (112-115 days as compared to 100-106 days).

Asdell (1916) reported that in the goat the age of the female has some in-

fluence on the length of gestation period, it is least for the first year conception and there is a gradual rise to a maximum of 151.3 days at about 6 years.

Rao (1966) reported on his observations on Malvi cattle that the average gestation period in primipara heifers extended to 283.83 days, in secondipara the average was 285.22 days and from 3rd gestation the average was 283.72 days. Rajalu and Rao (1966) did not find any significant influence of sequence of calvings on length of gestation period in Ongole cattle. Rao (1966) reported that the gestation period of primiparus Ongole heifers was 286.52 days, 288.27 days for secondipara and 288.36 days for the third conception. Hadi (1966) reported that in Deoni cattle the length of gestation period is maximum during the second gestation period and minimum during the third. This was statistically significant. Dange (1969) observed that in Gir breed the gestation length tends to increase upto the third gestation and then to decline upto the 5th gestation after which the trend was uneven. Ponkshe (1969) also observed similar trend in Gir breed. Kohli and Suri (1957) reported in Harijana cattle a gestational length of  $289.1 \pm 1.3$  days for primipara. There was a gradual increase during the 2nd and the third gestation after which considerable fluctuations were observed between 288.5 to 308.5 days. The differences were significant.

Choudhuri and Sinha (1951) reported in Thari cattle that there appears to be a tendency of slightly longer gestation periods upto the 7th calving i.e. about 10 to 12 years of age and thereafter

cows and that the male calves were carried about 2 days longer than the females Rajalu and Rao (1966) reported that in Ongole cattle the sex of calf had a significant effect on gestation period. Male calves were carried to  $289.16 \pm 6.18$  days and the female calves to  $287.64 \pm 6.40$  days. Rao (1966) reported from his observations on Malvi cattle that the male calves were carried for 288.56 days and female calves for 284.28 days. Littlewood (1937) reported that in Kangayam cattle the mean gestation period was  $288.6 \pm 0.30$  days for male calves and  $284.1 \pm 0.26$  days for the female. In Ongole cattle he reported the mean gestation period of  $289.8 \pm 0.32$  days for male calves and  $288.5 \pm 0.30$  days for the female calves. In Sindh cattle he has reported the mean gestation period of  $286.3 \pm 0.41$  days for male calves and  $284.5 \pm 0.38$  days for the female. Hadi (1966) reported in Deoni cattle a mean gestation period of  $285.26 \pm 0.59$  days for the male calf and  $284.49 \pm 0.49$  days for the female. The difference was highly significant. Kohli and Suri (1957) reported that in Haryana cattle, the mean gestation length for male calf was  $291.75 \pm 0.87$  days and  $289.68 \pm 0.94$  days for the female ones, the differences being significant.

Choudhuri and Sinha (1951) reported in Tharparkar cattle that the mean gestation period for male calf was  $288.50 \pm 0.46$  days and for female calf  $285.80 \pm 0.48$  days. The difference between the sexes of  $2.7 \pm 0.65$  days was significant.

Rao and Murari (1956) reported in Murrah buffaloes that the sex of foetus did not appear to have a significant effect on length of the gestation period.

Arunachalam *et al* (1952) reported in Murrah buffaloes that sex of the calf

had no influence on the length of gestation period. Ragab and Asker (1951) reported that in Egyptian buffaloes the males are carried longer as compared to females. Maymone (1945) however reported that the gestation period is shorter in case of the male calves. Bhattacharya *et al* (1967) reported in large white pigs that the correlation between the gestation period and sex ratio was not significant. Singh *et al* (1958) reported in Tharparkar cattle that the mean gestation period for male calves was  $289.49 \pm 0.46$  days and  $287.24 \pm 0.48$  days for the female calves, the difference of 1.76 days between males and females was not significant. El Dessouky and Rakha (1964) reported that in Egyptian buffaloes the sex of calf did not have significant effect on the length of gestation period. Domniqués (1961) reported that in buffaloes in Brazil no significant differences have been observed in the length of gestation period due to sex of the foetus.

Forbes (1967) reported in Scottish halfbred ewes that sex of foetus had no significant effect on the gestation period of the dam.

Nambiar and Raja (1962) reported in Murrah buffaloe cows in Kerala, the mean gestation period of  $304.89 \pm 0.49$  days. The  $304.31 \pm 0.67$  days for the male and  $305.56 \pm 0.71$  for the females. The differences due to the sex of the foetus were not significant.

Knoop and Hayden (1934) reported a slight increase in the duration of gestation with the age of dam upto 6 years of age. The average increase is 2.5 days. Brackel *et al* (1952) reported that the mean gestation length of 5 year old cows significantly exceeded that of 2 year old cows by 1.5 days.

Howell and Rollins (1951) reported a slight effect of age of mare on the period of gestation.

Sane *et al* (1969) in their observations on a Gir herd which was partly stallfed and partly on pasture observed that the average gestation period extended to  $281 \pm 1.9$  days. The average duration of pregnancy in primipara was  $270 \pm 1.87$  days, that of the secondipara  $276 \pm 1.26$  days and from the third conception onwards the length of gestation period was observed to be constant round about 284 to 285 days. They recorded a gestation period of  $287 \pm 2.83$  days in stallfed Sindhi cows and the duration of gestation was rather variable from calving to calving.

Sane *et al* (1969) recorded an average gestation period of  $311 \pm 0.35$  days in stallfed Surti buffaloes. The length of gestation period was slightly variable and there was no consistency from calving to calving denoting that the age of the dam had apparently little or no effect on the duration of pregnancy.

fluence on the length of gestation period, it is least for the first year conception and there is a gradual rise to a maximum of 151.3 days at about 6 years.

Rao (1966) reported on his observations on Malvi cattle that the average gestation period in primipara heifers extended to 283.83 days, in secondipara the average was 285.22 days and from 3rd gestation the average was 283.72 days. Rajalu and Rao (1966) did not find any significant influence of sequence of calvings on length of gestation period in Ongole cattle. Rao (1966) reported that the gestation period of primiparus Ongole heifers was 286.52 days, 288.27 days for secondipara and 288.36 days for the third conception. Hadi (1966) reported that in Deoni cattle the length of gestation period is maximum during the second gestation period and minimum during the third. This was statistically significant. Dange (1969) observed that in Gir breed the gestation length tends to increase upto the third

there is a tendency for shorter gestation periods. Arunachalam *et al* (1952) reported in Murrah buffaloes that the sequence of conception has no effect on the length of gestation period.

#### Effect of Age of the Dam on Gestation Period

Forbes (1967) reported in Scottish halfbred ewes that the gestation length decreases with age from 3rd to 5th pregnancy and then increases to the eighth

Ragab and Asker (1951) reported that in Egyptian buffaloes the calving sequence of the dam and her age have no significant effect on the length of the gestation period.

El Sheikh (1967) reported in Egyptian buffaloes that the sequence of calvings had no effect on length of gestation

Nambiar and Raja (1962) reported in Murrah buffaloes in Kerala that the sequence of calvings was found to be responsible for the differences in gestation periods which were highly significant. The gestation period for the second calving was 302.90 days and was the shortest as against 310.00 in the seventh calving which was the longest. (Table 26).

Table 26

#### DURATION OF GESTATION PERIOD IN FARM, LABORATORY AND WILD ANIMALS

Animal	Gestation period (in days)
<b>Cattle (Dairy breeds)</b>	
Holstein Friesian ...	278-280
Jersey ...	277-280
Dairy Short horn ...	281-282
Brown Swiss ...	288-291
Ayrshire ...	277-278
Guernsey ...	282-285
Swedish Red & White ...	284
Red Dane ...	285
Red Sindhi ...	288
Sahiwal ...	285
Tharparkar ...	287
Gir ...	284
Hariana ...	288
Rathi ...	286
Kankrej ...	289
Ongole ...	287
<b>Cattle (Beef breeds)</b>	
Aberdeen Angus ...	273-282
Hereford ...	283-286
<b>Cattle (Draft breeds)</b>	
Kangayam ...	288
Amrit Mahal ...	287
Hallikar ...	290
Khillar ...	287
<b>Buffaloes</b>	
Murrah ...	310
Surti ...	313
Nagpuri ...	305
Marathwada ...	309
Egyptian buffalo ...	316
Thailand buffalo ...	325
Philippine buffalo ...	332
Bulgarian buffalo ...	316
<b>Horses</b>	
Thoroughbred ...	338
Arab ...	337
Belgian ...	335
Shire ...	310
Stallion x Ass (Hinny) ...	350
Jack x Mare (Mule) ...	355
Ass ...	363
Zebra ...	315
<b>Sheep</b>	
Southdown ...	110
Dorset ...	111
Shropshire ...	116
Hampshire ...	145

Animal	Gestation period (in days)
Rambouillet	... 150
Merino	... 150
Corriedale	... 149
Goat	... 148-156
Deer	... 200-210
Swine	... 105-126
Dog	... 60- 63
Cat	... 56- 65
Siamese Cat	... 63- 69
Camel (Bactrian)	... 333-434
Camel (Dromedry)	... 315-350
Camel (Two humped)	... 406
Elephant (Indian)	... 615-650
<i>Laboratory animals</i>	
Rabbit	... 30- 32
Guinea pig	... 63- 70
Mouse	... 10- 20
Rat	... 20- 23
Hamster	... 16- 19
Mink	... 42- 53
Ferret	... 42
Monkey	... 139
Gorilla	... 258
Chimpanzee	... 227
<i>Wild animals</i>	
Lion	... 105-112
Tiger	... 105-113
Leopard	... 90
Fox	... 51- 52
Kangaroo	... 38- 40
Bear	... 208-210
Wolf	... 63
Rhinoceros	... 330-348
Hippopotamus	... 237
Giraffe	... 435
Bison	... 270-276
Beaver	... 90
Elk	... 245
Squirrel	... 23- 40
Reindeer	... 225
Walrus	... 330
Whale	... 360

### Litter size in sheep

Nichols (1926) observed that young ewes pregnant for the first time at about 18 months, gave a much smaller lambing percentage than multiparous ewes. Wridt (1925) observed that in sheep fertility rose upto the fifth year. Kelley (1937) has shown that in Merino ewes the percentage of twins rises to a maximum between 5 and 10 years of age and then falls to a low level in later years. Breed differences were however common. Lapryn (1935) observed that in early maturing breeds of sheep, the maximum fertility may be attained at 3 years and in late maturing breeds by 5th to 6th year.

### Litter size in sows

Stewart (1955) observed that gilts farrowing for the first time at 320 days of age on an average produce one piglet less than those farrowing at 410 days. Those doing so at 410 days on an average produce half a piglet more than those farrowing at 365 days of age. Lush and Molln (1942) on their extensive data in pigs have observed that the number of piglets farrowed rises to about 2 years of age remains at high level till about 4½ years, after which it gradually declines. Experimental evidence has shown that in young growing sows, average corpora lutea detected in the ovaries vary from 10 to 14 while as in the fully grown adult sows an average of 20 have been recorded. McKenzie (1956) found that sows bred for the first time late in their life averaged only 5.6 young as compared to 8.4 young for those bred at the normal time.



was  $7.89 \pm 0.04$  and average weaning weight as  $73.65 \pm 1.72$  kg

### Factors affecting litter size

Mikewa (1967) reported that gilts had smaller litters than sows but the percentage of dead piglings was roughly the same. The percentage of runts in litters of gilts was only about half of that in the litters of sows. The seasonal mortality rate was highest in litters of sows inseminated in the Autumn. In both sows and gilts litter size was greatest when inseminations were carried out in November and December but this effect was not significant.

In litters farrowed by gilts a significant size effect was observed on the total number of piglings (live or dead) born per litter but not on the number of live born pigling. In litters farrowed by sows however a highly significant boar effect was found on the number of live born piglings and the number of live and dead piglings. Significant sire effects on the percentage of dead piglings and runts were observed in litters of sow but not in those of gilts. In both sows and gilts there was a tendency for larger litters to contain more dead piglings and more runts.

Plocek (1967) reported in pigs that there was no significant difference in the number of live piglings born per litter when the dam was first mated at less than 8 months or at 16 to 18 months. The percentage of still born piglings and total pigling mortality was highest in litter of females first mated at less than 8 months and at 18 months or more of age.

### PLACENTA AND PLACENTATION

Reinoldus Columbus (1516-1559) was the first to introduce the term placenta.

As defined by Mossmann (1937) it is an intimate apposition or the fusion of foetal organs to the maternal tissues for physiological exchange. These extra embryonic membranes or the placental membranes can be differentiated into the amnion, allantois and serosa. The amnion surrounds the foetus. The outer most membrane is the serosa which lies in contact with the endometrium. The inner layer of allantois is fused to the amnion and outer layer to the serosa forming chorioallantois. The allantois is continuous with the bladder via urachus which is located in the umbilical cord. The chorioallantois brings the foetal blood vessels in close contact with the umbilical arteries and veins which are located in the connective tissue between the allantois and chorion.

### Types of Placentation

Types of placentation vary in different mammals depending upon the degree of fusion between maternal and foetal tissues, the internal structures of uterus and the size of the litter. The classification of mammalian placenta is based on several criteria.

The nature and purpose of placenta is to facilitate the transfer of metabolic materials between the mother and foetus. This involves the maternal placenta on one hand and the foetal placenta on the other. The degree of union between these two structures varies in all mammals and accordingly the placentae have been variously classified depending upon the

- (a) separation of maternal tissue at birth
- (b) morphological shape and size of the placenta and
- (c) histological structures involved in the separation of the vascular

systems of the foetus and the mother.

### Classification of the placenta

(a) Depending upon the separation of the maternal tissue at birth.

Endometrial relations of the placenta varies greatly and may involve merely a close apposition of the foetal and maternal tissues called as 'Apposed placenta' (non desiduate) or their intimate fusion called as 'Conjoined' (Desiduate) placenta. In the former, there is no intimate fusion of the tissues and their separation is easily effected at birth without the removal of the maternal tissues. Such placentae are known as non desiduate as seen in cow, mare, ewe, and sow. Whereas, there is an intimate connection between the uterine wall in the Desiduate type and a considerable amount of maternal tissue is shed off along with the placenta, as seen in bitch and cat.

(b) Depending upon the morphological shape and size of the placenta.

Placentae are classified on the basis of their shape and size. Morphologically four main types are recognised.

(1) *Diffuse*: In some angulates like sow and mare, the villi are spread over the entire chorion. This is the diffused type of placentation, the area of which is more expansive.

(2) *Cotyledonary*: In ruminants such as cattle, buffalo, sheep, goats and deer, the villi are grouped in well spaced prominent rosettes called as cotyledons separated by smooth area of chorion (Fig. 67a, b, c).

(3) *Zonary*: In carnivores like bitch and cat, the villi occupy a girdle like band about the middle of chorionic sac.

(4) *Discoidal*: The villi of insectivores, bat, rodents and primates are limited to one or two disc shaped areas.

(c) Depending upon histological structures involved in the separation of the vascular systems of the foetus and the mother:—

The histological classification is dependent upon the number of tissue layers that separate the two vascular systems, viz. those of the foetus and mother. This classification includes three types.

#### (i) *Epithelio chorial*:

In this type of placentation the chorionic villi are spread over the placental surface. It is of diffuse type and seen in sows and mares. The chorioallantois and uterine wall are held together due to the increased vascularity of these tissue. Six layers of tissues separate the foetal and maternal blood. They are (1) foetal vascular endothelium (2) connective tissue of chorio allantois (3) serosal ectoderm (4) uterine epithelium (5) connective tissue of the endometrium and (6) endothelium of the maternal vessels.

Areolae are the specialised areas of chorio allantois which lie against the uterine glands and are meant for absorption of uterine milk in sows whereas endometrial cups are present in mares. In the mare the endometrial cups also contain a hormone gonadotropin by about 50th day of pregnancy.

#### (ii) *Syndesmo chorial*:

This type of placentation is found in cattle and sheep and is characterised by the absence of any uterine epithelium overlying the caruncles. The caruncles are convex in the cow and concave in the ewe (Fig. 68) and their number varies from 70 to 120 in the cow and

90 to 100 in the ewe. The number and degree of development depends upon the nutritional status of the mother.

**Cotyledons** — The foetal cotyledons are attached to the caruncles through villi and all these structures together are known as placentome. In cattle they develop in 4th or 5th week of pregnancy around the foetus and extend towards the end portion of chorioallantois in nongravid horn by about 12 to 13 weeks. Enlargement in the size occurs, those in the centre of gravid horn being larger than those at the extremities. During growth they become round, pedunculated and mushroom like. Their degree of development is much less in the non gravid horn.

**Chorionic villi** — Chorionic villi appear in the form of parallel ridges opposite each uterine caruncle. This occurs in ewes by about 27th day. Primary villi are formed from the prominences on these ridges. This primary villus branches and re branches. The villi are slender during early stages but later increase in size, maximum length being seen in latter part of gestation. The villus consists of a vascular mesenchymal tissue surrounded by cubical trophoblastic and giant cells. Through the erosion of uterine epithelium intimate contact develops between serosa and stroma of the endometrium.

### (iii) *Endothelio chorial*

This type of placentation is found in carnivores in which species a marked intimacy of foetal and maternal tissues in the region of villous girdle is observed. Uterine mucosa is eroded and the endothelium of blood vessels is bare around which the syncytial chorionic epithelium is packed.

### (iv) *Haemo chorial*

In rodents, insectivores, bats and arthropods a still more intimate placental union is observed in which complete erosion of superficial uterine mucosa occurs. The chorionic villi are directly bathed by maternal blood that issues from the eroded blood vessels. At birth the placenta tears away as a unit.

### (v) *Haemo endothelial*

In some higher rodents like rats, guinea pigs and rabbits, the actual intermingling of the maternal and foetal blood circulation occurs. Flexner (1954) from his electron microscopic studies considered that placenta in these species was of a haemochorial rather than haemoendothelial type.

### Vascularisation of placenta

The nutrition of foetus is of a haematrophic type and therefore the vascularisation of placenta is important. The uterine arteries and veins which run through the caruncles and crypts are coiled but those of foetus penetrating the villus do not reveal much coiling. The capillaries connect the axial arterial branch. The foetal villi in the crypts are surrounded by maternal tissue and they branch into small loops which resembles a leaf like pattern (Tsutsumi and Hafez, 1966).

### Functions of Placenta

The placenta has to perform three major functions viz transport, storage and biosynthesis. The mother has to supply the essential factors required in the synthesis of large amount of new tissue in addition to maintaining herself, since pregnancy is predominantly an anabolic process. In the prenatal life, nutrients are supplied in four stages. In

the first i.e. cleavage stage in farm animals the cleaved egg obtains its nutrition from its own deutoplasm which is temporary.

In the second, the blastocyst stage, blastocyst absorbs fluids and nutrients from the uterine luminal fluid. In the third stage, the vitelline circulation helps the histotrophic nutrition during implantation. In this stage phagocytosis occurs which engulf fluid unaltered fats and tissue debris. In the fourth stage which is a major route of prenatal nutrition in farm animals, absorption of nutrition takes place by the allantoic circulation in the placenta after the formation of placentomes.

Detailed description of role of the placenta, in the transport of nutrition, while maintaining and permitting the pregnancy is as follows:

### **Transport of Nutrients**

At the junction of chorion and endometrium even though there is no direct contact of blood of the foetus and dam, the circulations are close enough to pass oxygen and nutrients from the maternal blood to the foetal blood and the waste products in the opposite direction. However, the placenta has selective permeability which protects the foetus against foreign material molecules as well as prevent transfers in opposite direction. Carbohydrates, proteins and fats as well as water, inorganic salts pass in one form or other from the dam to the foetus. The mechanism involved in this transfer is quite interesting. According to Hammon's "Partition of nutrients" theory, the foetal tissues get priority over the maternal tissues and it has been observed that the foetus grows even when the mother is loosing condition. The transfer of metabolic products through the placental

membranes is by simple diffusions such as movement of molecules from high to low concentration. Secondly by some active transport certain ingredients can even be pumped from low to high concentration. Thirdly by phagocytosis i.e. by engulfing the food particles and fourthly pinocytosis i.e. engulfing the water molecule. The ease of the passage depends upon the solubility of the nutrient components.

### **Transport of Gases or Respiration**

The oxygenated blood from the mother is carried to the foetus through the tributaries of umbilical veins whereas the unoxygenated blood from the foetus is carried by the tributaries of umbilical artery. The two gases namely oxygen and carbon dioxide pass through the membranes by diffusion, the process of which is regulated by the blood pressure and its flow depending upon the foetal requirements.

On account of the low pH in the placenta, the oxygenated haemoglobin from the mother is readily disassociated thereby releasing oxygen for foetal haemoglobin. Carbon dioxide is readily transferred from the foetus to the mother. The placenta thus acts like a lung.

### **Transport of water**

Water moves very freely between mother and foetus. Of the total substances absorbed by the foetus, 77 per cent is water. However it is interesting to note that the water moves from mother to foetus against the osmotic gradient and low concentration of plasma proteins.

### **Transport of Inorganic nutrients**

Of the inorganic nutrients, sodium is much restricted in the passage through

placenta The iron content is much higher in the foetus which is stored in liver spleen and bone marrow The transference of trace elements like copper through the placenta is readily effected This element also accumulates in the liver Although manganese is found in the foetal liver it does not accumulate Calcium and phosphorus according to Newland *et al* (1960) enter the foetal blood against a concentration gradient Depletion of calcium and phosphorus reserves from the mother occurs in cases of low plane of nutrition

Of the total sugar content of the foetal blood 70-80 per cent is fructose whereas glucose predominates in maternal blood Probably glucose is formed in the placenta and stored in foetal liver which serves as a reserve energy source Placenta is not permeable to fat but the fatty acids and glycerol pass freely Vitamin A D and E are obstructed by placenta and hence their concentration in the foetus is much lower Since the water soluble Vitamins are synthesised in rumen their concentration in foetus has not been studied Placenta is also permeable to all hormones particularly gonadotropins steroids and insulin The gonadotrophic hormones pass easily through the placenta causing enlargement of foetal gonads

### Barrier

Placenta is impermeable to particulate matter like bacteria and even to large molecules However this barrier is selective allowing certain substances to pass and retaining others contrary to ordinary osmotic procedures

### Synthesis

Hormones such as oestrogen, progesterone and gonadotrophin are produced in considerable amounts by the

placenta Similarly certain food stuffs are synthesised

### Excretion

Fluid waste products like urea of the foetal metabolism escape through the placenta to the mother which thus has a kidney like function

The foetal membranes are the means of protection, nutrition and excretion during the prenatal life These are expelled at birth They consist of yolk sac amnion allantois and chorion

### YOLK SAC

This is a primitive structure observed in the early embryonic period in all the domestic animals except poultry It atrophies completely after few weeks It is elongated structure in the cow, ewe and sow Before the formation of amnion the blastocyst and the yolk sac perform limited placental functions They provide nutrients and take care of early embryo The nutrients in the form of uterine milk are absorbed by the blastocyst and the yolk sac

The uterine milk contains glycogen fructose leucocytes RBCs, fat globules inorganic salts immune bodies from lymph and probably other nutrients This is secreted by the uterine glands of the endometrium under the influence of progesterone

### AMNION

As the development of embryonic disc continues the embryo takes the tubular form Simultaneously, the amniotic margin follows this underfolding In this fashion the line of attachment becomes limited to the ventral body wall and then decreases in relative extent as it bounds the umbilical area

With the development of umbilical cord, the amniotic portion near the umbilicus appears as a covering layer. The amnion becomes a thin, tough, transparent and nonvascular membrane. The amnion is observed only in mammals, birds and reptiles while it is absent in fishes and amphibia.

The inner lining is an ectodermal epithelium while outer layer is a mesodermal connective tissue. The amniotic cavity enlarges rapidly and a clear, colourless mucoid, amniotic fluid accumulates.

Amnion is formed at about 13 to 16 days after conception in cow, sheep and mare and little earlier in the pig, dog and cat. The embryo is suspended in the amniotic fluid. In the bovines, amniotic plaques or pustules are present on the inner surface of amnion. These are small 1.5 to 12.5 mm, irregular in shape, flat, white and raised epithelial thickenings which are usually seen between third to seventh month of pregnancy and disappear late in gestation. They are rich in glycogen. The causes and significance of these plaques is not known. Neither there is any relation to bacterial or viral origin nor are they of any inflammatory origin. The quantity of amniotic fluid varies in farm mammals being about 3500 ml. (3000 to 5000) in the cow, 3000 to 7000 ml. in mare, 400 to 1200 ml. in the goat, 40 to 150 ml. in the sow and 8 to 30 ml. in the dog and cat. The volume varies considerably in different species and even within individuals. The amniotic fluid is secreted by the amniotic membrane and the skin of foetus. But its exact origin is unknown. It is alkaline in nature and contains pepsin, proteins, fructose, fat, salts and diastatic and lypolytic ferments (Markle, 1944). The bovine and swine foetus

swallow much of this fluid during the stages of pregnancy and hence the quantity remains fairly constant. Urine from foetus is also excreted into the amniotic cavity through the urethra.

The amniotic fluid provides a watery medium in which embryo develops freely, without any effect of distortion due to surrounding rigid structures. Thus it serves as a protective water cushion which absorbs jolts. It prevents adhesions of the foetus to the amniotic membrane. The amniotic fluid aids in the dilatation of cervix at the time of parturition. During this time, the amniotic fluid becomes more mucoid and of slippery consistency which serves as a lubricant that assists the expulsion of foetus. The amniotic fluid equalises the pressure and permits the change of foetal posture. The role of amniotic fluid with regard to the detoxification or retention of foetal excretions and secretions is not known.

### ALLANTOIS

Allantois arises as an outpocket of the hind gut from the splanchnopleura. Its outer layer is rich in blood vessels forming the umbilical artery and the vein. With the progressive development allantois grows, enlarges and extends into the amniotic cavity (i.e. with the true and false amnion). This cavity contains allantoic fluid and is formed completely by about 24 to 28 days after fertilization in domestic animals. It extends along the whole length of foetal membranes except in the undilated apices of the chorionic vessels. Since the latter is devoid of blood vessels it atrophies, becomes necrotic and are known as necrotic tips of allanto-chorion. Between 60 to 90 days of pregnancy in cow, calcium deposits are seen on the allanto-chorion in the form of white streaks,

which disappear afterwards. The waste products from foetal kidneys and bladder pass through the umbilical cord through the urachus and are stored in allantoic cavity. The allantoic fluid is clear, watery, amber coloured with a slight alkaline reaction and contains proteins (albumins), fructose, urea, creatinine and inorganic salts. According to Wislocki (1935), Richter and Gotze (1950), the quantity of allantoic fluid in different species is as follows: cow 9500 ml. (4000-15000 ml.); mare (4000-10,000 ml.); goat (500-1500 ml.); sow (10-150 ml.), bitch (10-50 ml.) and cat (3-15 ml.).

In the allantoic cavity are found irregular shaped masses or bodies called as 'hippomanes'. These are amber coloured, pliable rubberlike with an amorphous bodies semisolid in consistency and varying between 0.3 cm to 4.00 cm in thickness. These are usually found floating in the allantoic cavity. Their origin and significance is not understood. No cellular structure or tissues could be differentiated histologically.

### CHORION OR ALLANTOIS CHORION

This is formed by fusion of the outer layer of allantois and the trophoderm or serosa which arises from somatopleura. This structure is in intimate contact with endometrium. It is highly vascular and carries out the interchange of gases, nutrients and wasteproducts between foetal and maternal circulations. This function starts from its formation. The allanto-chorion is the foetal placenta and its development is dependant upon the requirements of the foetus. Solids and bacteria are impermeable through this structure, except in diseases of chorion. However certain viruses and larvae of parasites can pass through this barrier. In cow, pig and

sheep, allantois is attached to the amnion at various places, giving the appearance of separate compartments. On this account the foetus is never born with intact amnion. In contrast to this, the amnion containing the foetus floats freely in the allantoic cavity in mare, bitch and cat, and the young one may be born with intact amnion or portion of it covering the nostrils and mouth. In cow, ewe, and sow the tips of chorion are necrotic being about 1 to 2.5 cm. long and 0.3 cm. wide. A portion of diffuse placenta or the chorion lying over the internal os of cervix in mare is known as 'cervical star'.

### Weight of the placenta and number of cotyledons in the cow

Subramanian (1961) recorded average weight of placenta of Haryana cow as 5.00 lb and average number of foetal cotyledons as 77.1 of which 41.8 were in gravid and 35.3 in non gravid portion. Purbey (1965) recorded the mean weight of foetal membranes as 4.7 lb (range 2.5 to 6.2 lbs.), the average number of cotyledons being 77 out of which 41.00 were in the gravid portion and 36 in non-gravid portion. Kadvekar *et al* (1968) reported mean weights of the placenta of Gir and Rathu cows as  $2.31 \pm 0.104$  and  $1.91 \pm 0.09$  kg. respectively.

Sonawane (1969) recorded the mean weight of the placenta in Rathu cows as  $1.82 \pm 0.11$  kg (range 1 to 3.00 kg). The average number of cotyledons was 84.05 out of which 48.81 were in the gravid and 35.24 were in the non-gravid portion while the placenta of Gir cow weighed  $2.42 \pm 0.35$  kg (range 1 to 3.50 kg), the average number of cotyledons being 77.60 —45.50 in gravid and 32.10 in non-gravid portion. In both breeds 58% of the total number of

cotyledons were in the gravid portion. He observed that weight of the placenta was 10% of body weight of new born calf.

Ponkshe (1969) reported 2.41 kg as the average weight of placenta of Gir cows (range 2 to 3.55 kg). The average number of cotyledons was 104 out of which 55.75 were in gravid and 49 were in the non-gravid portion. The average length of greater curvature was 229.6 cm. and that of lesser curvature 145.24 cm.

Dange (1969) reported average weight of placenta of Gir cow as 2.173 kg (range 1.465 to 3.270 kg). The average number of cotyledons was 78.41 out of which 44.08 were in the gravid and 34.66 in non-gravid portion. He further observed that 56.21% of the total number of cotyledons were in gravid portion and the weight of the placenta was 12% of the body weight of new born calf. The mean length of greater curvature was 221.00 cm (range 167 to 254 cm) and that of lesser curvature 138.3 cm (range, 102 to 151 cm).

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# Chapter 20

## Pregnancy Diagnosis

Diagnosis of pregnancy at an early stage is important. A regular check up is necessary to confirm if the animals have settled to services and if not, to investigate the causative factors for subfertility or infertility. A Veterinarian should have good knowledge of the physiopathology of reproduction and the experience of having examined a number of cows and buffaloes both non-pregnant and also the pregnant ones in various stages of gestation. He should also acquaint himself further by examining a large number belonging to different age groups in several breeds, from calving to calving to distinguish changes in the size, shape and feel of the uterus. He should have good knowledge regarding differential diagnosis — to differentiate normal pregnancy from foetal mummification, maceration, pyometra, mucometra, hydrometra and tumours.

In carrying out pregnancy diagnosis, following points should be taken into account:

**History.**

Preparation of the animal for examination.

Precautionary measures.

External examination — visual and manual.

Mammary development.

Vulval and vaginal changes during pregnancy.

Gynaec examination by rectal palpation in large animals.

Biological and chemical tests.

Radiographic examination.

Differential diagnosis of Pregnancy.

### History

On a cattle breeding farm or city stables attempts should be made to keep breeding records which would give certain information regarding various aspects of the breeding performance. The records should give the following information:

- (i) Birth date to estimate age.
- (ii) Age at which the first oestrus was exhibited.
- (iii) Service dates, and details of service (natural or A.I.) and name of the sire.
- (iv) Date of first and subsequent calvings.
- (v) Previous history, such as the date of last calving, whether delivery was normal, placenta expelled in good time and details of any treatment.
- (vi) Date of last service.

In the usual course, absence of oestrus after mating in all domestic animal species, except in the bitch and the cat, is considered as probable indication of pregnancy. It is also true that in a number of pathological conditions, oestrus may be absent though the animal is not pregnant. A certain percentage of cows and buffaloes exhibit oestrus on one or more occasions even though they are pregnant. It is risky to serve such animals. One should not rely on history only and actual examination of such cases is very necessary to diagnose if the heat is gestational. Examination at six weeks stage would give a clear idea in majority of the cases. All cases which repeat to service after an interval of about two to three months need careful examination.

### Preparation of the animal for examination

The animal should preferably be examined after milking so that the excitement does not affect the milk production. The cow or buffalo should be restrained properly in the standing position. Since majority of the Indian cows and buffaloes are of excitable temperament, it is better to secure them in a trevis for examination. If cows are fairly quiet, it is customary to examine them in the byre-proper by securing the hindlegs. There is every danger of the animal slipping during examination particularly if the floor is slippery. Normally it is not necessary either to fast the animal or to withhold water a few hours earlier to examination. On no account the animal should be cast for pregnancy examination.

### Precautionary measures

A Veterinarian has to prepare himself suitably so as to have ease and safety in

carrying out the examination. Considerable importance is attached to protective clothing which comprises of rubber or plastic overall, full sleeves and gumboots, which is easy to clean and sterilise. A goggle to protect the eyes is necessary. Before putting on the protective clothing nails should be trimmed. Protective clothing minimises the possibility of to and fro transmission of infection when a Veterinarian has to visit a number of farms on the same day.

### External examination

*Visual:* The animal should be carefully observed with regard to physical condition.

In heifers improvement in general condition and slight abdominal enlargement may be noticeable from the third month of gestation. In the cow, the enlargement is very variable depending upon the breed, number of calvings and pendulous belly. Abdominal enlargement should not be relied upon as the only method of diagnosis. It is just an aid. In majority of cows the abdominal distension may be noticeable from the 6th month onwards. The enlargement is specially marked on the right side and easily detectable in the heifer than in the cow. In the obese cow, it may not be detectable until late in gestation.

*Manual — Abdominal ballotment:* In palpating the abdomen, a closed fist should be pushed gently but firmly below the right flank, midway between the last rib and the hind limbs. The pressure is released quickly, keeping the palm against the flank. It is possible to feel the foetus by applying pressure on the flank from the seventh month onwards. Quick release of pressure may be

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freshly put to pasture. In constipated animals, rectal mucosa is dry and prone to injury. During summer months when there is acute shortage of water and green fodder, rectal mucosa gets dry and bleeding may occur due to manipulation.

Difficulty will be encountered in examining obese cows per rectum owing to fatty deposits around their sexual organs. In hilly cattle and small breeds like Dangi, Khillar and Nimar (India) difficulties are usually experienced in carrying out rectal palpations.

#### **Guidelines in rectal palpation of genital organs**

The pelvis and the internal genital organs can be thoroughly palpated per rectum. The pelvic or the pubic brim, the iliac shaft, the obturator and the lesser sciatic foramina can serve as guidelines in recognising the various segments of the pelvis during the rectal examination. In non-pregnant animals, the vagina can be felt immediately ventral to the rectum as a soft band running straight anteriorly towards the pelvic brim. The cervix is felt at the anterior end of vagina as a firm cylindrical continuation, somewhat nodular in structure, on the mid-line of the pelvic floor. In heifers, the cervix is at a distance of about 25 cm from the anus. Whereas in cows it is about 35 cm. Cervix is freely movable and can be easily grasped in the hand. The vaginal opening of the cervix (os uterus externum) can be palpated with the thumb. In heifers, the uterus is found entirely within the pelvic cavity whereas in the cows its position differs, depending upon the breed, size and parity. The position even in non pregnant condition will vary from animal to animal. Heifers which have

not reached puberty upto 2 to 3 years of age, show small cord like uterine horns.

The size, shape and consistency of the cervix varies with the breed, age, stage of the reproductive cycles, number of calvings and the presence of any abnormalities. In non-pregnant cows, it is about 6 to 10 cm long and having the diameter of about 3 to 4 cm. The cervix in cows becomes hypertrophied after each pregnancy and the enlargement is prominently marked at the posterior end.

Anterior to cervix, body of the uterus is felt as a comparatively soft structure. In heifers the uterine body is about 2 to 3 cm. in diameter. In cows, the diameter is twice or even more.

At the junction of the intercornual ligaments both the cornua bifurcate and each may be palpated forwards, outwards, downwards, backwards and upwards. The approximate length of the cornua from the bifurcation varies from 15 to 40 cm. and the diameter is about 2 to 3 cm. in the non-pregnant animals. The horns are felt tightly coiled.

The ovaries are located posterior and slightly lateral to the apical ends of the uterine cornua and can be palpated by following the horns right from the cervix. The size, shape and consistency of the ovaries is variable according to the breed, number of calvings and the stage of oestrous cycle. Corpus luteum in various stages of development would be located in either of the ovary. Regressed corpora lutea of various sizes also can be felt. The ovary which does not contain the corpus luteum is oval in shape, smooth and firm in texture. The approximate length of ovaries varies from 1.5 to 2.5 cm, thickness is about 1.0 to 2.0 cm and breadth 1.5 to 2 cm. A few

followed by rebound of the foetus and it is markedly felt by the palm of the hand.

### **Mammary development during pregnancy**

In primiparous heifers, from about the fourth month onwards, some enlargement of the udder is noticeable. At this stage a thick, brown, very sticky secretion, pre-colostrum can be pressed out from the teats. The enlargement of udder is well definable but the extent of volume is only marked during the last month of pregnancy. In non-pregnant heifers the feel of udder tissue is soft. Due to pregnancy, the hormonal changes influence the mammary tissue. Oestrogens will lead to the proliferation of the alveolar and the duct systems as a result of which the udder tissue can be distinguished as a well palpable mass having good consistency. The feel is variable between individuals and stages of pregnancy. The udder is distended to its maximum during the last 3-4 days before parturition with the presence of colostrum which is yellowish white and viscous in consistency.

In heifers and cows where steaming up is practised, the onset of colostrum and milk secretion may take place as early as 3 to 4 weeks before parturition. Certain amount of oedema is also noticeable during the last week or so of gestation. The extent of oedema is however variable.

In cows, which have conceived while in lactation, the mammary changes are not apparent in early pregnancy.

### **Technique of Rectal Palpation**

It is possible to carry out a thorough examination of the internal genital

organs and the pelvis of the cow per rectum (P.R. examination).

A lubricated gloved hand is slowly introduced into the anus when slow dilation of the anal sphincter helps further entry into the rectum. The gentle introduction of hand into the rectum leads to peristalsis and defaecation reflex. Backraking may be done if necessary, giving no chance for the air to rush into the rectum. Once the hand is inserted in the rectum it should not be removed, but the faeces only should be pushed out by the palm to avoid ballooning of the rectum. The distension of the rectum with air, will make it tense and this interferes with proper examination. If attempts are made to examine when the rectum is ballooned, it is likely to cause rectal injury bleeding and even perforation. The rectal distension can be overcome by grasping the most anteriorly located contracted folds of the rectum and pressing the air out by gentle backward movement of the folds by repeated to and fro movements of the fingers at the dorsum antero-posteriorly, simultaneously exerting pressure on the floor of the rectum. The ballooning can thus be overcome. Rectal haemorrhage may occur due to:—

- (i) Forcible manipulation.
- (ii) Manipulation in the distended rectum due to aspirated air.
- (iii) Manipulations during peristaltic waves when the animal is straining.
- (iv) Sharp finger nails.
- (v) Manipulations involving long periods.

Rectal haemorrhage may occur in cows that have an oedematous rectum. In diarrhoea and enteritis, rectal oedema is frequently observed. Rectal oedema is also noticed in cows which are

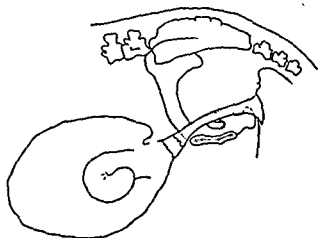


Fig. 69b. Progressive enlargement of uterus in cows during pregnancy. (6 to 8 weeks).

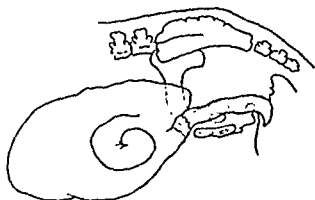
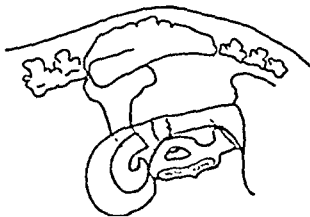


Fig. 69c. Progressive enlargement of uterus in cows during pregnancy. (12 to 20 weeks).



- (iii) Increase in the size of the uterus.
- (iv) Typical thinning of the wall of the gravid cornua.
- (v) Slippery feel of the foetal membranes during early pregnancy.
- (vi) Feel of the cotyledons.
- (vii) Feel of the conceptus and foetal bump.
- (viii) Enlargement of the middle uterine arteries and fremitus.

#### Conception to 4 weeks

During this stage there is no palpable enlargement of the uterus. The corpus luteum develops until about the 16th day and then becomes firmer. If the animal conceives the corpus luteum does not regress from 18th to 20th day as during normal cycle, but it continues to develop, becomes firmer and deeply embedded in the ovary. The animal does not repeat to service on the 21st day. Tentative diagnosis is only made on the assumption, that there was a fertile service, the animal did not repeat and the corpus luteum continues to grow and becomes firmer in one of the ovaries. Re-examination should be done after a fortnight to confirm the diagnosis.



small follicles may be present on the surface of the ovary. A follicle may develop and become ripe at any point. Ripe follicles measure 0.5 to 1.0 cm in diameter and it is felt as a hemispherical, blister-like protrusion on the ovarian surface. Care is required in manipulating the ovaries since a matured Graafian follicle is likely to be ruptured on palpation. Corpus luteum about 2 cm in size may be felt as a soft protrusion during the first few days after ovulation. It gradually develops further and at the end of 2 weeks it is felt as a firm body well embedded in the ovary and having a diameter of about 2 to 3 cm. If conception has not occurred the corpus luteum slowly shrinks and regresses between 18 to 21 days.

The ovary is freely movable. When the fingers are directed downwards from the ovary they would enter into the ovarian bursa which has a fine thin texture and 2 to 3 fingers can be admitted in its pouch. The fallopian tubes pass backwards from the apex of the uterine cornua to the ovary on the lateral walls of bursa. The fallopian tubes owing to their fine structure cannot normally be palpated unless they are enlarged as in salpingitis, hydrosalpinx and pyosalpinx.

### Rolling up of uterine cornua

It is not possible to palpate all parts of the uterus in its position since slight touch immediately leads to coiling and the cornua cannot be felt properly along their entire length. One should therefore practise retraction or rolling up of the uterus first in non-pregnant animals and then in early pregnant ones. During early pregnancy as that of 5 to 7 weeks correct diagnosis is only possible on thorough palpation with rolling of the uterine cornua.

At 5 to 8 weeks stage of pregnancy, it may be difficult to palpate cornua except certain portions anterior to the body of the uterus. Fluctuation is not well marked. In such cases it is necessary to roll up the uterus.

Both ovaries should be examined to find which one is bigger and having corpus luteum. If pregnant the corresponding horn would be bigger in size. Locate the cervix and pull the uterus up and back into the pelvis and should then quickly get hold of the intercornual ligaments with the finger and lift the uterus higher up dorso-caudally in the pelvic cavity. Pull both the horns back in the pelvis, palpate each one carefully when difference in the asymmetry can be judged. Fluctuation and thinning of the uterine wall may also be felt. Both the uterine cornua should be palpated along their entire length.

In certain cases it is difficult to roll up the uterus. Under the circumstances the non-pregnant horn corresponding to the small ovary should be pulled back. It will thus be possible to palpate the pregnant horn and detect asymmetry and fluctuations.

When palpating the genital organs per rectum the following points would aid in the diagnosis (Fig. 69 a, b, c, d).

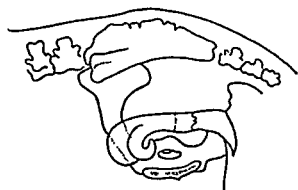


Fig. 69a Progressive enlargement of uterus in cows during pregnancy (4 to 6 weeks)

the foetus can be felt by pressing against the dorsal wall of the uterus and quickly releasing the pressure. Double uterine wall may be felt with ease before the cotyledons start developing from 12 to 16 weeks but this feel is not necessary in view of the positive enlargement.

*Fremitus* can be felt at about the 4th month which continues to be progressive until the full term. This is due to the hypertrophy of the middle uterine artery on the gravid side as a tense cord about 1 cm in diameter. The artery is located by palpating the pelvic wall at about the level of middle third of the shaft of the ilium; when the artery is compressed between the fingers and the thumb, a vibratory sensation is felt which is termed '*fremitus*'.

The vagina becomes pale and the cervix gets more tilted. By the end of the 20th week, the long axis of cervix assumes inclination away from its normal horizontal position.

### Diagnosis of twins and multiple births

During early period of gestation, when the ovaries are still palpable per rectum, the presence of two corpora lutea, either in the same ovary or one on each ovary will indicate twin pregnancies. Between 5 to 8 weeks before extensive enlargement occurs, a separate set of developing foetal membranes may be detected in each cornua in case ovulations have occurred from corresponding ovaries. Such bilateral enlargement of the uterus is likely to be confused with pyometra. Proper examination is therefore necessary to find that there is thinning of the uterine wall, slipping of the foetal membranes, fluctuations and tenderness. In case of monozygous twins the diagnosis would be difficult since there is only one corpus luteum in the ovary and two sets of foetuses developing in the uterine cornua.

### Differential Diagnosis of Pregnancy

In the diagnosis of pregnancy, it is likely that structures like the urinary bladder, rumen, free end of caecum and

cept in cases where conception has occurred in the other cornua during subsequent pregnancy.

The ovary corresponding to the gravid cornua will show a well developed firm corpus luteum deeply embedded.

At 6 weeks stage, enlargement is well defined with fluctuations and slipping feel of the membranes. For an accurate diagnosis the uterus should be rolled up and cornua well palpated. The vaginal mucosa looks palepink. The cervix is found centrally placed upto 6th week of pregnancy and the entire uterus is intra-pelvic in majority of cows.

#### 6 to 8 weeks

At the end of the 6th week, the cornua corresponding to the ovary with the corpus luteum enlarges to about 2 to 3 times its normal diameter at its most pendant point. The enlargement tapers away from this point on both sides. The whole of the enlargement can be held in the palm of the hand after the uterus is rolled back. Thinning of the uterine wall continues and fluctuations become marked. Slipping feel of the foetal membranes is marked particularly in the heifers.

At six weeks there is no enlargement of the non gravid horn. The gravid horn enlarges very rapidly from 6 to 8 weeks and disparity between the gravid and non gravid cornua becomes marked. The pregnant horn at 8 weeks stage is approximately 6 times greater in volume. Its content are tense and double wall is felt. Some enlargement and tenseness of the body of the uterus occurs at 8 weeks stage but very little palpable enlargement of the non gravid cornua is seen. In majority of primipara and multipara, the uterus is partly intra pelvic and

partly extra-pelvic in position. The uterus can still be palpated with ease from all sides. No foetus can yet be felt.

The vaginal appearance is similar to that observed in earlier stages of pregnancy.

#### 8 to 12 weeks

During this stage, the non-gravid cornua enlarges to about double its normal size, the gravid horn further becomes enlarged and tense. The uterine wall becomes very thin and due to its increasing weight, the uterus becomes extra-pelvic and hangs on the brim of the pelvis. It is still possible to palpate it from all sides. The ovary can be located and the corpus luteum of pregnancy can be felt. The double wall feel is easily palpated. At the end of this stage, ballotment of the foetus through the uterine wall is at times possible.

The vaginal mucous membrane is pale to pink. The cervix is pulled forward and becomes tilted on the pelvic brim due to the weight of the gravid uterus. On rectal palpation, vagina is felt as a tight band drawn forward. The vulval lips are also drawn in, due to the pull exerted on the vagina.

#### 12 to 20 weeks

There is rapid enlargement of the uterus during this period and it becomes entirely abdominal at about the 17th week. Vaginal tension is progressive. The cervix is located beyond the pelvic brim. The dorsal surface of the uterus can be well palpated. After about 15 weeks, it is not possible to palpate the uterus from all sides. The uterine wall becomes less tense. From about the 16th week, the cotyledons can be felt as cork like bodies floating on the surface of water. From 16th to 20th week,

of uterus unicornis and also in other developmental anomalies of the uterus, cervix or vagina and in chronic cases of cystic ovaries causing cystic degeneration of the uterine wall.

The above conditions differ from pregnancy due to the absence of feel of foetal membranes and the foetus, lack of fremitus, and failure of progressive development of the uterus as during normal gestation. Pathological conditions such as hydrops amnii and hydrallatois are likely to be confused with mucometra and hydrometra, particularly in early stages but this can be differentiated by the tense feel of the overdistended uterus, presence of foetus and cotyledons.

#### 5. White Heifer Disease

In the White Heifer disease certain forms are characterised by retention of uterine secretions. The uterine enlargement may be unilateral and simulates early pregnancy. Differential diagnosis is possible on the ground that there is no double uterine wall and the lumen of the vaginal canal just beyond urethral orifice is not patent. The vagina may be occluded by a permanent hymen and the cervical canal may be absent. This occlusion is detected when uterine catheter is passed.

#### 6. Tumours

Tumours may cause confusion in pregnancy diagnosis. The tumours most commonly confused with pregnancy are lymphosarcoma, granulosa cell tumours of the ovaries and fat necrosis in the mesentery. Depending upon the type, situation and consistency of the tumour, it is possible to differentiate from pregnancy.

#### 7. Chronic Endometritis

The differential diagnosis can be made on the grounds that in chronic endometritis there is absence of uterine contents, no thinning, no slipping feel of foetal membranes and absence of cotyledonary feel. The cervix is mostly enlarged. In uncomplicated cases, oestrous cycles may occur. In chronic endometritis the uterine discharges invariably contain flakes of pus.

#### 8. Incomplete or delayed involution of uterus

Incomplete or delayed uterine involution may occur due to several factors and when such cases are offered for examination, without any history, confusion arises in proper diagnosis. Within 3-4 days after parturition, the uterus is supposed to involute very rapidly but this may be delayed due to hormonal imbalance, pathological conditions and dystokia. The lochial discharge contained in the uterus may be confused with enlargement of pregnancy at various stages. It can only be differentiated on the grounds that the uterine wall is thick. The quantities of accumulation of lochial discharge is variable and likewise the difference in fluctuations. The lochial discharge which is occasionally voided soils the perineal region. Repeated examinations will show that in context to pregnancy, there is a consistent reduction in the size of the uterus. Hypertrophied cervix and fremitus may give deceptive feeling. Straining which is absent in pregnancy is occasionally observed.

#### Other methods of Pregnancy diagnosis in the cow

Methods for the diagnosis of pregnancy based on the detection of changes in the composition of blood and urine

### 1. Pyometra

The size of the uterus in pyometra may approximate to various stages of pregnancy due to accumulation of pus in variable quantities from 0.5 to 20 litres. A corpus luteum on the ovary is present and oestrus does not occur. Slipping of the foetal membranes is not felt. There is absence of cotyledons. Fremitus may or may not be present. The enlargement should be estimated if it corresponds to the service date. Usually, confusion may arise at 5 to 9 weeks after last service. Pyometra commonly is a sequelae of retained afterbirths and metritis wherein there is an intermittent discharge of pus from vagina. Pregnancy may occur even in Trichomoniasis but the foetus is destroyed during early stage, the foetus and foetal membranes macerate, resulting in pyometra. In such cases the cervical seal of pregnancy may remain undisturbed for a long period. The uterine walls are comparatively thick and heavy. There is a lack of tone to the uterus and the fluid in the uterus is thick to semi-fluid consistency compared to normal placental fluids giving the typical 'doughy' feel on uterine palpation per rectum. Asymmetry of uterine cornua as in pregnancy is absent, the uterine enlargement is bilateral with the presence of a single corpus luteum and the absence of double wall feel which will serve to differentiate pyometra from twin pregnancy. Differential diagnosis can only be made on account of the absence of foetal membranes or the pus tending to accumulate in the apical portions of both cornua with no dorsal bulging as is palpated during early pregnancy. In normal pregnancy, repeated examinations will indicate progressive enlargement of the gravid cornua while as in pyometra, the en-

largement more or less remains of the same size and is uniformly bilateral. In pregnancy, the cervical mucus appears sticky and tenacious but in pyometra the cervical mucus is slimy and moist.

### 2. Mummification (Haematic mummification)

In the bovines mummification of the foetus may occur during 3rd to 8th month of pregnancy.

History of service but failure of the increase in size of the foetus or abdomen, failure of udder development and failure of parturition are suggestive of mummified condition. There is absence of cotyledons and foetal fluids, the uterine wall becomes thick and tightly adherent to the hard foetal mass (foetal mummy). Fremitus is absent. Corpus luteum can be palpated on the corresponding side of the ovary and the cervical seal remains intact.

### 3. Maceration

In this condition, the symptoms are similar to those of pyometra, except that the foetus dies in utero after about the fourth month of gestation, accompanied by bacterial invasion resulting in maceration of the soft structures of the foetus except the bones. On rectal palpation, the foetal bones lying in the lumen of the uterus will give a 'crepitan' feel. Foetid, purulent, discharge in variable quantities is voided from the vagina from time to time. In certain cases where the bones are locked up in the cervix very hard irregular feel is experienced with sharp bony protrusions. (Fig. 70).

### 4. Mucometra and Hydrometra

This may occur secondary to an imperforate hymen in the defective cornu

of uterus unicornis and also in other developmental anomalies of the uterus, cervix or vagina and in chronic cases of cystic ovaries causing cystic degeneration of the uterine wall.

The above conditions differ from pregnancy due to the absence of feel of foetal membranes and the foetus, lack of fremitus, and failure of progressive development of the uterus as during normal gestation. Pathological conditions such as hydrops amnii and hydrallatois are likely to be confused with mucometra and hydrometra, particularly in early stages but this can be differentiated by the tense feel of the overdistended uterus, presence of foetus and cotyledons.

## 7. Chronic Endometritis

The differential diagnosis can be made on the grounds that in chronic endometritis there is absence of uterine contents, no thinning, no slipping feel of foetal membranes and absence of cotyledonary feel. The cervix is mostly enlarged. In uncomplicated cases, oestrous cycles may occur. In chronic endometritis the uterine discharges invariably contain flakes of pus.

## 8. Incomplete or delayed involution of uterus

have proved unsatisfactory. Several tests carried out for gonadotrophins and oestrogens in the blood and urine on different test animals and urine test for pregnandiols have proved unsatisfactory.

### Foetal Electrocardiograms (F.E.C.G.)

The technique was used for cattle, sheep and horses. The electrocardiographic diagnosis of bovine twin pregnancy is unsuccessful after five months of gestation. It is hoped that clinical application of F.E.C.G. may prove to be of great help for the diagnosis of late foetal mortality, mummification and multiple births in the cow. For diagnosis of triple and quadruple pregnancies use of an electro cardiograph with two or more channel system is desirable.

### Foetal Heart beats

In twin foetuses heart beats are generally similar but at times one foetus may show tachycardia (very rapid heart beats) while in the other foetus the heart beats are normal. Foetal arrhythmia is usually observed in tachycardia.

### Diagnosis of Pregnancy in the Buffalo

Procedures adopted for the diagnosis of pregnancy in the buffalo are more or less the same as that in the cow. It has to be remembered that the duration of gestation period in the buffalo is 10 months and 10 days. The gestational development of the foetus and progressive gain in weight is in accordance with the birth weights in different breeds of buffaloes. Whenever there is no history of services, the Veterinarian will have to ascertain the period of gestation according to the size of the foetus on palpation per rectum.

In the buffalo, restraint is easier as compared to the cows. Buffaloes can be

examined in the stable or on the standings proper in the open, just by securing their heads. Buffaloes do not kick like cows. All examinations should be done in standing position. In case of excitable buffaloes, forelegs are tied by means of a rope in standing position to check their movements.

In the actual examination per rectum, the following points would serve as guidelines. It is experienced that in the buffaloes the rectal wall is rather tense and rectal mucosa is delicate as compared to that in cows. Bleeding is commonly observed if care is not taken during backraking. Rectal palpation of the genitalia has to be done quickly and gently, else the animal is likely to strain and forcible palpation may lead to rectal bleeding. Good amount of lubrication of hands is therefore essential.

In majority of the buffaloes, either in heifers or in adults the uterus in non-pregnant condition is invariably found on the floor of the pelvis. Observations made by Sane *et al* (1968) and Kaikini (1971) showed that hypertrophy of the cervix and uterus, as it occurs from calving to calving in the cow, which results in enlargement and size of the cervix and uterus, is not commonly marked in the buffalo. In consequence of which, even in pluriparous buffaloes, the non pregnant uterus is found on the floor of the pelvis while as in pluriparous cows, the uterus is found partly intra-pelvic and partly extra-pelvic or completely extra-pelvic in cows which have given more than 6 to 8 calvings.

### Pregnancy Diagnosis in the Mare

It is necessary to carry out pregnancy diagnosis in the mare as a routine in case breeding is to be done systematically. This is in view of the fact that a

certain number of mares do exhibit oestrus even if they are pregnant and if pregnancy diagnosis is not done in good time, there is every chance that they will be served again, resulting in complications. It is experienced that some mares do not repeat to oestrus in good time even if they have not settled to service. This gives an erroneous impression that the mare once served is pregnant. The Veterinarian should collect as much history of the mare as possible such as the last foaling date, service date etc. prior to rectal examination. It is essential to take into account the age, general condition of the mare, previous breeding history, date last seen in oestrus, reaction to the teaser before and after breeding, to serve as a guideline for proper diagnosis.

#### **External signs of pregnancy in the mare**

nancy beyond ninth month of gestation. Enlargement of the mammary glands is noticeable in the last month of gestation. A very distinct distension of the teats is marked at about 2 to 4 days prior to parturition. In most mares, 4 to 48 hours before foaling, waxing of the teats due to expression of colostrum is noticed. However, great variations are not uncommon in advanced pregnancy. Oedema of the abdomen, just anterior to the udder may also occur. In the mare, relaxation of the pelvic ligaments in advanced pregnancy is not so marked as in the cow. External ballotment of the foetus detectable in the cow in advanced pregnancy, is not apparently marked in the mare owing to the thickness and tenseness of the abdominal wall. During the last month of pregnancy, foetal movements may be seen in the abdominal wall after the mare drinks cold water. Preceding



It is necessary to secure the mare in a trevis or against bails of hay or a gate. Withholding the ration a day prior to the examination may help in avoiding back pressure during the rectal palpation.

The veterinarian should put on the protective clothing and lubricate his hand with non irritant non-greasy lubricant such as the soft soap. In mares backracking is most essential as dung pellets may complicate the examination particularly that of ovaries. The tail should be properly secured. The hand is then slowly introduced in the rectum to palpate genitalia of the mare.

#### Location of ovaries

The examiner should be fully conversant with the anatomical structures and their relationship involved in pregnancy examination of the mare. There are certain land marks which would serve as a guide such as the pelvic girdle with its typical characteristics. The ovaries serve as a second landmark owing to their relatively constant site and its distinguishing features. On introduction of the hand in the rectum, examination is usually begun by location of one of the ovaries. It is the usual experience that the left ovary is the easier to reach and locate as compared to the right one.

In non-pregnant mares as well as those in early pregnancy, the ovaries are found located in the sub-lumbar region, at a distance of 5 to 10 cm anterior to the upper third of the shaft of the ilium. The iliac bone is located first and thereafter the ovary can be easily traced. The ovaries have a typical oval and irregular form and a firm consistency. The size and shape of the ovary is dependant on the age, body confirmation, season, stage of the oestrous cycle

and the reproductive status of the mare. In majority of the mares, the left ovary is comparatively larger than right. The average size is 5 to 8 cm in length and 2 to 4 cm in width and height.

A beginner may get confused while palpating per rectum due to the faecal balls in the small colon and proximal part of the rectum. The ovaries are firm and are distinguishable from the faecal balls which are soft in consistency and disintegrate on pressure. In case ovaries cannot be located in the prescribed position, it is suggestive of advanced pregnancy.

The landmarks which usually serve as guidelines during pregnancy diagnosis are in general the same as observed in the cows.

#### 1. Progressive increase in the size of uterus

In early pregnancy increase in size is typically circumscribed and does not involve the entire horn as in the cow. In most cases, pregnancy is established in the right cornua. In early pregnancy the enlargement can be located slightly above the junction of the horn with the body. As the pregnancy progresses, the uterine contents expand and the body (Corpus uteri) becomes the main site for the future development of the conceptus. This is in contrast to the observation in cows and buffaloes where the conceptus develops in either of the cornua and not in the body.

#### 2. Fluctuations

The formation of foetal fluids is associated with the increase in size of the foetus. This causes circumscribed ventral distension of the uterus. Thinning of the wall of the distended segment is clearly palpable.

### 3. Foetal membranes

In the mare, the placentation is of a diffuse type and as such, the typical feel of the cotyledons during pregnancy as in the cow is absent. In early stage of pregnancy upto 50-60 days the bulging of the uterus is entirely due to the amniotic vesicle. After 50-60 days of pregnancy, the foetus becomes palpable and the amniotic vesicle becomes indistinct.

### 4. Hypertrophy of the blood vessels

During progressive stages of pregnancy, the demand for blood supply constantly increases resulting in the hypertrophy of uterine blood vessels with a marked increase in the size of utero-ovarian and mid-uterine artery. Caudal uterine artery is also hypertrophied. Fremitus is perceptibly felt after about 150 days of pregnancy.

### 5. Location of the uterus

There is a progressive change in the position of the uterus depending upon the increase in its size associated with weight gain. Upto 60th day of gestation, the uterus is found intrapelvic, except in pluriparous and old mares. At about the 90th day of pregnancy the uterus hangs down on the brim of the pelvis and continues to descend progressively until it reaches the abdominal floor by the 6th or 7th month. Thereafter, a gradual ascent of the foetus begins.

also exerted on the broad ligaments so that they get displaced forwards, inwards and downwards. The ovaries are also pulled forwards and displaced in the same direction. By the end of the descending stage, the ovaries are practically found as low as the level of the pelvic brim.

### Palpation of uterus and ovaries in non-pregnant mares and during early stages of pregnancy

It is necessary for a Veterinarian to get himself thoroughly acquainted with the characteristic feel of non-pregnant uterus and ovaries by palpation per rectum in a large number of mares of various breeds and age groups. Location of the ovaries in the high sub-lumbar region, either indicates uterus in a non-pregnant state or an early pregnancy. In such a condition, one should carry out a thorough examination of the entire uterus to differentiate size, shape or tone in different parts. The ovaries should be located first and thereafter one should grasp the utero-ovarian ligament to trace the apex of the horn. Retraction or rolling should not be done. It is not possible to finish palpation of uterus in one attempt due to peristalsis and straining. In case of straining, one should discontinue the palpation and re-examine subsequently.

### Diagnosis of pregnancy during various stages of Gestation

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and the reproductive status of the mare. In majority of the mares the left ovary is comparatively larger than right. The average size is 5 to 8 cm in length and 2 to 4 cm in width and height.

A beginner may get confused while palpating per rectum due to the faecal balls in the small colon and proximal part of the rectum. The ovaries are firm and are distinguishable from the faecal balls which are soft in consistency and disintegrate on pressure. In case ovaries cannot be located in the prescribed position it is suggestive of advanced pregnancy.

The landmarks which usually serve as guidelines during pregnancy diagnosis are in general the same as observed in the cows.

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### 6. Position of the ovaries

During pregnancy, change in the position of the uterus also involves corresponding changes in the broad ligaments and the ovaries. During gradual descent of the gravid uterus, tension is

also exerted on the broad ligaments so that they get displaced forwards, inwards and downwards. The ovaries are also pulled forwards and displaced in the same direction. By the end of the descending stage, the ovaries are practically found as low as the level of the pelvic brim.

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It is necessary for a Veterinarian to get himself thoroughly acquainted with the characteristic feel of *non-pregnant* uterus and ovaries by palpation per rectum in a large number of mares of various breeds and age groups. Location of the ovaries in the high sub-lumbar region, either indicates uterus in a non-pregnant state or an early pregnancy. In such a condition, one should carry out a thorough examination of the entire uterus to differentiate size, shape or tone in different parts. The ovaries should be located first and thereafter one should grasp the utero-ovarian ligament to trace the apex of the horn. Retraction or rolling should not be done. It is not possible to finish palpation of uterus in one attempt due to peristalsis and straining. In case of straining, one should discontinue the palpation and re-examine subsequently.

### Diagnosis of pregnancy during various stages of Gestation

In pasture bred and newly purchased mares, a good check-up is essential to find if they are pregnant and if so the stage of pregnancy. It is possible to estimate the stage of pregnancy with greater accuracy during the first 3 months since the periodical changes can be well differentiated.

*4 weeks or 30 days pregnancy:*

The non-pregnant uterus has a soft velvety flaccid feel. At about the 15th day of pregnancy, the uterus becomes turgid.

The earliest period at which pregnancy diagnosis is possible is at thirty days stage when a characteristic circumscribed spherical enlargement of the uterus measuring 2.3 cm in diameter is palpable. In the mare, this enlargement projects ventrally in the lower third of the gravid horn close to the body of the uterus. There is a marked tension in the enlarged segment of the uterus with a good tone. Palpation per rectum may lead to its contractions.

Cervix is located on the pelvic brim and in front of it, the corpus uteri bifurcates in the right and left horns at right angles. Difficulty is usually felt in picking up the cornua with fingers for palpation. In some mares, there is a downward curve at the base of the horn which might be mistaken for enlargement of early pregnancy. A careful palpation of both the horns from apex to the cervix and at their bifurcation will help in the differential diagnosis. The volume of the foetus, membranes and fluids is too small in the gravid uterus due to which there is no conspicuous bulge.

There is presence of one corpus luteum in the ovary corresponding to the gravid horn and follicles of various sizes in both the ovaries. During the first 3 days following ovulation the presence of corpus luteum can be felt as a soft area in the ovulation fossa. The corpus luteum of the mare does not project over the surface of the ovary as in the cow and its presence is only recognisable from the enlargement in the

size of the ovary. At 30th day of pregnancy, the vaginal mucus membrane becomes much paler than at any other time during various stages of oestrous cycle. Day (1940) mentioned that there is a pearly appearance of the vaginal mucous membrane and covered by fine network of small blood vessels.

*35 days :*

At this stage the enlargement attains the size of 3 to 4 cm and the uterine wall of the enlarged segment becomes thinner with clearly palpable fluctuations.

*45 days :*

The enlargement increases in size to 5 to 7 cm in length and 5 cm in diameter. It attains an oval form and the enlargement reaches upto the junction of the horn and the body of the uterus. At about 40 days the enlargement simulates the size of urinary bladder, which has to be differentiated. On careful palpation, the bladder can be distinctly felt as a separate structure lying below the uterus.

*48 to 50 days :*

The progressive enlargement now assumes distinct oval form and it now involves the body of the uterus. It measures 7 to 8 cm in length and 6 to 7 cm in diameter and occupies about two-thirds of the uterine horn and one-third of the body of the uterus. Fluctuation is clearly palpable. In majority of mares, the uterus at this stage is strictly intra-pelvic.

*60-65 days :*

At this stage it assumes the size of a foot-ball. The enlargement measures 12 to 15 cm in length and 8 to 10 cm in diameter which occupies the uterine horn while, approximately half the

enlargement is located in the body of the uterus.

### 90 to 100 days :

The size of the enlargement measures 20 to 25 cm in length and about 12 to 16 cm in diameter involving the entire body of the uterus. The gravid horn begins to descend over the pelvic brim. It is not possible to retract the uterus and the ventral bulge cannot be grasped. On ballotment of the enlarged uterus, the foetus can be easily detected.

### 3 to 5 months :

The progressive descent of the gravid uterus pulls the broad ligament downwards, forwards, and medially. Its anterior edge assumes the position where ovaries were present in the non-pregnant state. The ovaries are also pulled close together further anteriorly and ventrally. Palpation of the uterus in various directions is still possible at this stage.

### 5 to 7 months :

From 5th month onwards, the arch of the dorsal wall of uterus cannot be palpated at or near the pelvic brim. The descent of the uterus is completed by the end of the 7th month. This is followed with increased tension on broad ligaments and further displacement of the ovaries. The foetus at this stage can be felt by ballotment. This is done by slowly compressing the dorsal wall of the gravid uterus and immediately releasing the pressure whereby foetal bump can distinctly be felt. Fremitus is distinctly felt.

### 7th month to parturition :

The entire uterus is fully descended into the abdomen. There is a gradual decrease in the tension from period to

period. Foetal parts such as limbs, head, ribs etc. are easily palpable.

### Vaginal changes during pregnancy

During oestrus the vaginal mucous membrane in the mare is bright pink, glistening and covered with ample fluid mucus. The cervix is relaxed and oedematous. During dioestrus, it becomes pale or slightly blanched. It looks dry and the cervix is constricted. A few weeks after the animal has settled to service, the mucous membrane becomes pale and dry and as the period of pregnancy advances, the mucus becomes sticky and viscous. The cervix is firm, constricted and the external orifice readily visible. In about 4 weeks time, the vaginal mucus becomes opaque, sticky, and of gummy consistency. The vaginal mucosa appears very pale. It is difficult to insert the speculum since the vaginal walls become adherent to each other. A thick mucoid seal of pregnancy is seen at the external os.

### Ovarian changes—during pregnancy

In relation to the changes that occur in ovaries during pregnancy, Cole *et al* (1931) have divided the gestation period in four stages.

*First stage :* This stage extends from conception to 48 days and is characterised by the presence of a corpus luteum in either of the ovary and follicles of different sizes in both. During this period, the blood serum has no effect on the ovaries of immature rats even in large doses.

*Second stage :* This period extends from about 48 days to 150th day of gestation. Follicles continue to appear and are luteinised enmass. As many as 10 to 15 follicles of 1 cm size may ap-

*4 weeks or 30 days pregnancy:*

The non-pregnant uterus has a soft velvety flaccid feel. At about the 15th day of pregnancy, the uterus becomes turgid.

The earliest period at which pregnancy diagnosis is possible is at thirty days stage when a characteristic circumscribed spherical enlargement of the uterus measuring 2-3 cm in diameter is palpable. In the mare, this enlargement projects ventrally in the lower third of the gravid horn close to the body of the uterus. There is a marked tension in the enlarged segment of the uterus with a good tone. Palpation per rectum may lead to its contractions.

Cervix is located on the pelvic brim and in front of it, the corpus uteri bifurcates in the right and left horns at right angles. Difficulty is usually felt in picking up the cornua with fingers for palpation. In some mares, there is a downward curve at the base of the horn which might be mistaken for enlargement of early pregnancy. A careful palpation of both the horns from apex to the cervix and at their bifurcation will help in the differential diagnosis. The volume of the foetus, membranes and fluids is too small in the gravid uterus due to which there is no conspicuous bulge.

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*60-65 days :*

At this stage it assumes the size of a foot-ball. The enlargement measures 12 to 15 cm in length and 8 to 10 cm in diameter which occupies the uterine horn while, approximately half the

From this point it gradually diminishes and by about the 150th day it can not be detected. The hormone is formed in the endometrial cups of the uterus in the pregnant mare and this peculiar formation of the uterine mucosa is present from 6th to 28th week of pregnancy.

The presence of the follicle stimulating hormone in the blood serum can be demonstrated by stimulating ovarian activity in immature rats or mice. This serves as a means of diagnosis of pregnancy during 45 to 100 days of gestation.

#### *The Ascheim Zondek (A.Z.) Test*

This test was first described by Ascheim and Zondek (1927) in women. They showed that the gonadotrophic hormones were excreted in the urine of pregnant women by 35th day after the last menstruation.

**Technique:** 2 to 3 immature female rats of about 22 days old are required for the test. 0.5 ml of serum from the suspected mare is injected subcutaneously daily for 24 days. Rats are killed 96 to 120 hours later and the uterine cornua and ovaries are examined for characteristic changes. In positive cases the horns increase in size 2 to 4 times with the presence of haemorrhagic spots or corpora haemorrhagica on the ovaries.

ed for this test. 2 ml of mare serum is injected intravenously in the ear vein. Laparotomy is performed 24 hours after the injection. The presence of corpora haemorrhagica in the ovaries and oedematous condition of the uterus indicates positive diagnosis.

**Mouse test:** Miller and Day (1938) found that the mouse test gives 90% accuracy for diagnosis of pregnancy in the mare between 60th and 100th day of gestation. Miller (1938) also found that at the 120th day of gestation, the mouse test gives about 80% accuracy.

#### **'Chemical Test'**

**Urine:** The presence of oestrogenic hormones in the urine of pregnant females was first discovered by Ascheim and Zondek (1927). At about the 75th day after conception the pregnant mare generally commences to excrete oestrone in her urine in appreciable quantities. This may be delayed until the 85th or 90th day. In exceptional cases, 120th day may be reached before mare's urine contains sufficient quantities to indicate positive reaction. From this time onwards the amount increases until 280th to 290th day after which it decreases until during the last month of gestation when the amount is very small.



pear forming solid corpora lutea. During this stage the blood serum of the pregnant mare contains a high level of chorionic gonadotrophins.

*Third stage* This period extends from the commencement of the sixth month and terminates at the end of the 7th month of pregnancy. The chief feature of this period is characterised by the regression of corpora lutea and a complete absence of large follicles.

*Fourth stage* This period extends from the beginning of the 8th month till parturition. During this stage the regression of corpora lutea is practically complete. Follicles if any are never bigger than 1 cm in size.

Available literature shows that in most mares conception takes place in the right horn. It is difficult to say if this is due to the ovum liberated from the right ovary or transuterine migration of ovum from the left ovary as the incidence of the latter is high in the mare.

### **Diagnosis of Twin and Multiple Pregnancy**

Twin and multiple pregnancies are rare in the mare. Twin pregnancy can be detected in most cases at about 40 to 45 days stage. On rectal palpation two amniotic vesicles or a ventral bulge in each horn is detected. During later stages twin foetuses are easily palpable. Twin pregnancies in the mare are usually followed by abortions during late stage of gestation. Mammary development well in advance before term may be an indication of twin pregnancy.

### **Differential diagnosis**

Diagnosis of pregnancy in the mare may get confused on account of several difficulties of various types. Distended

urinary bladder may be confused with pregnancy of 70 to 100 days. The distended right colon or its fluctuating pelvic flexure might be confused with a gravid uterus from 90 to 120 days of pregnancy.

Pathological conditions such as pyometra might cause confusion in pregnancy diagnosis. Pyometra is rare in the mare. The uterine wall is thick, heavy and the fluid contents of the uterus are sluggish, which is in contrast to normal pregnancy. The mucus membrane of the vagina and the cervix is pale and gummy.

Tumours, mummification of foetus, foetal maceration and mucometra due to congenital anomalies are of rare occurrence. Cystic degeneration of the uterine wall might be confused with pregnancy. In such a condition the uterine wall becomes very thick and boggy, resulting in the drag of the uterus into the abdominal cavity.

### **Other methods of pregnancy diagnosis in the mare**

Diagnosis of pregnancy in the mare is also possible either by biological test or biochemical test.

### **'Biological Test'**

#### *Blood serum*

Cole and Hart (1930) were the first to discover the presence of gonadotrophic factor rich in follicle stimulating hormone in the blood serum of the pregnant mare. Since then it is known as pregnant mare serum hormone or PMS. It is first detected between the 37th and 42nd day of pregnancy in the mare and reaches its maximum level between the 50th and 88th day. Peak concentrations may persist to 106th day.

Tentative pregnancy diagnosis in ewes is done by shepherds by balloting at the base of udder in ewes. In ewes that have conceived an enlargement is felt at the base of the udder.

Recently ultrasonic foetal doplers are used to detect pregnancy by recording heart sounds of the foetus.

### Test for Gonadotropins

Examination of either the serum or urine for presence of gonadotropins has not given successful results for diagnosis of pregnancy in the ewes (Cowie, 1918).

### Test for oestrogens

Except a solitary reference, attempts to detect oestrogen in the blood and urine of pregnant ewes have led to negative results (Cowie, 1918).

## Pregnancy Diagnosis in the Sow

### Physical examination

In the sow it is not possible to carry out pregnancy diagnosis by physical examination such as per rectum, per vaginum or through external palpation of the abdominal wall.

Ultrasonic detection of the gravid uterine pulse per rectum is said to give reliable results between gravid and non-gravid uterus compared to the earlier practice of applying detector to the abdominal wall (Fraser, 1968).

non-pregnant sows. It is only present in the urine during oestrus. Observations show that oestrogens can be detected in large amounts in the urine of sows between the 23rd and 31st day of pregnancy and just after parturition. Except during the 4th week, lesser quantities are detected than at the other times of pregnancy (Cowie, 1918).

### Chemical Methods

Chemical methods give reliable results in determining the presence of oestrogens in the sows urine between 20th and 33rd days of pregnancy and also after 72nd day (Roth *et al*, 1911).

### Pregnancy diagnosis in the bitch

In most of the domestic animals non-recurrence of oestrus after mating is taken as a sign of pregnancy. In the bitch however this may not be true. In this species if true pregnancy is not established the oestrus period is usually followed by pseudo-pregnancy changes.

### Physical examination

after the Benzol layer is collected and 10 ml of concentrated sulphuric acid ( $H_2SO_4$ ) added to it and heated in a water bath at  $80^\circ C$  for 5 minutes. It is then cooled. In case the mare is pregnant, a green fluorescence develops. With the urine of non-pregnant mares, no colour change would occur. Positive reaction first appears at about the 120th day of pregnancy.

#### *Phenosulphuric acid test*

Mayer (1944) devised this test which is based on the Kober calorimetric test for oestrogens.

#### *'Mucin Test'*

Kurosowa (1931) described microscopical examination of the vaginal mucous in the pregnant mare as a method of pregnancy diagnosis. The method has been further developed by Miller and Day (1938).

**Technique:** A little of the tenacious mucous is drawn from the region of the os uteri either by sucking in glass pipette or by using a vaginal speculum and a swab. The smears are made on a number of glass slides. After fixing with alcohol and allowing it to dry, the smear is stained for 20 minutes with Delafield's haemotoxyline and then washed. The affinity of the mucous for stain is much greater in the pregnant mare than in the non-pregnant one. In positive cases the smear will appear dark blue in colour. When examined under the 2/3rd objective, very distinct globules of mucin are seen in the thinner parts of the smear. In the field where there are large number of cells, examination should continue with the 1/16th objective. A number of pregnancy cells will be observed if the mare is pregnant. These are columnar epithelial cells which have the appearance of bent tintacks, the dark staining

nucleus being situated a little behind the point. The cytoplasm of these cells stains very faintly. A number of other cells are also present but if there are globules of mucin a number of pregnancy cells and a small proportion of other cells leads to a positive diagnosis.

Miller and Day (1938) recorded their results on the test which show an ascending accuracy from 77.6% in the 20-40 days period to an average of 94.8% from 70th day onwards.

### **Pregnancy diagnosis in the Sheep**

#### **Physical examination**

It is not possible in the ewe to carry out rectal palpation for pregnancy diagnosis on account of the small size of the anal sphincter, which does not permit the introduction of human hand. In late stages of gestation however, the abdominal enlargement and mammary development gives a good indication in making the diagnosis. External abdominal palpation of the foetus is possible only during the latter third of the gestation period i.e. after about  $3\frac{1}{2}$  months. In primiparous ewes, mammary hypertrophy is marked at about the commencement of this period and precolostrum can be expressed from the teats.

#### **Radiographic examination**

This method for diagnosis is rarely necessary. It is possible to diagnose pregnancy and the number of foetuses from 90 days onwards by radiographic observations of the foetal vertebral columns. Benzie (1951) using radiographic technique obtained 96% accuracy in ovine pregnancy diagnosis from 43rd day of gestation. It is experienced that in heavy ewes the method may not give accurate results owing to the presence of massive mass of maternal tissue (Ford *et al* 1963).

*Conception to 18 days:*

No detectable changes in the size of the uterus are palpable during this period, except that there is a good tone to the uterus at 18 to 21 days.

By the end of the third week, the distended foetal membranes are palpable as well marked ovoid fluid filled swellings in the uterine horns. The size of the enlargement will vary according to the bitch. The approximate average diameter of each such swelling will be 1 to 1.5 cm.

*21-30 days:*

This is considered to be the optimum period for early pregnancy diagnosis. The enlargement gradually becomes spherical in shape and measures about 2 to 3 cm in diameter.

During this period, there is a rapid increase in the size of the uterus and it comes in contact with the abdominal wall. The foetal membranes lose their tensity and elongate as a result of which the entire uterus becomes enlarged and separate dilatations of the cornua can be felt. Actual palpation of foetuses is not possible upto this stage.

Movement of the foetuses is occasionally seen against the flank between 50 to 55 days of gestation in the bitch.

*55 days to term:*

During the last fortnight of the gestation period, it is possible to palpate foetuses per rectum, if the bitch is supported on her hind legs.

**Biological tests**

Cowie (1918) reported that biological tests have not given any successful results with the blood and urine samples of pregnant bitches.

1. *Gonadotropic hormones:* No traces in the blood, urine, amniotic fluids and milk are detected.

2. *Oestrogenic hormones:* There is some evidence that oestrogenic hormones in minute quantities may be present in the urine of the pregnant bitch after the third week of pregnancy, but not in perceptible quantities to be of any great value in the diagnosis. Harrop (1958) reported that oestrogens have not been detected in the blood of pregnant bitches.

3. *Progesterone and pregnandiol:* No traces of either have been detected in the urine, or in the placenta of pregnant bitches.

### Vaginal examination

Vulval enlargement which occurs during oestrus may persist for a variable part of pregnancy but this may be misleading since it also occurs during pseudo-pregnancy. Cornified cells can be found in the vaginal smears (Hancock and Rowland, 1949). Earlier, there is abundance of mucus during the first half of gestation which later on diminishes and the cornified cells can thereafter be detected in vaginal smears. However, this is not very reliable, since similar cell picture is also present during other periods.

### Mammary development

In primiparous bitches, changes in the mammary glands are more marked than those in pluriparous ones. From about the 20th day of pregnancy, a slight discrete zone of swelling is noticeable around the teats which progressively increases, throughout the gestation period. The abdominal and inguinal teats become enlarged earlier. Between 35 to 45 days, the teats further increase in size and become turgid. After 45 days they become more enlarged and soft. From about 50 to 55 days of pregnancy, the mammary glands further increase in size and become oedematous. Such changes in the mammary development are also noticeable in pseudo-pregnancy.

In multiparous bitches, no mammary enlargement is apparent till the last week of pregnancy. Few days before whelping, thin secretion can be expressed from the teats. The onset of true milk secretion however commences immediately after parturition. In few cases multi gravidæ, milk may appear at the teats even a week before whelping.

### Abdominal Palpation

Abdominal palpation is the most reliable method of pregnancy diagnosis in the bitch. However the successful diagnosis is dependant on the following factors:

*Temperament of the bitch:* In a nervous bitch, there will be tenseness of the abdominal muscles on palpation, which makes diagnosis difficult.

*Size of the bitch:* In a small bitch, it is easier, since palpation is possible with one hand alone.

*Condition of the bitch:* In fat bitches, it is extremely difficult to make pregnancy diagnosis by abdominal palpation.

*Number of foetuses in utero:* When only one or two foetuses are present, they are usually well forward and the detection becomes difficult.

*Period of Gestation:* Abdominal palpation is possible only after certain stage of pregnancy.

A good deal of practice is necessary to get oneself acquainted in the abdominal palpation for diagnosing pregnancy in the bitch. Much depends on the skill and experience of the examiner and the stage of pregnancy.

The examiner should first try to identify the bifurcation of the uterus at the pelvic brim, just above the bladder and ventral to the colon. Palpation of each horn should be done by pressing it between the fingers as it extends upwards, forwards and outwards to the sublumbar region, ending just close posterior to the kidney. In small breeds, the examination can be done with the fingers of one hand but in larger one and those which are obese, palpation should be done with both hands.

# Chapter 21

## Infertility in Cows and Buffaloes

### Anaestrous condition or failure of oestrus

Anoestrus is a period of sexual rest in which there is complete absence of sexual cycles with no manifestations of heat. There is temporary inactivity of both the ovaries. Anaestrous condition is observed during pregnancy and for a short period as that of two to three months after parturition. During other times, it is normal for the cow class, to show regular cyclical activity. When there is absence of periodic manifestation of oestrus, with absence of follicles of corpora lutea the condition is known as "true anoestrus". Ovarian hormones determine cyclical behaviour but ovarian activity is in turn stimulated and sustained by pituitary gonadotropic hormones. When there is deficient output of pituitary gonadotropins, anoestrous condition may occur. On account of high level of progesterone, secretion of FSH may be suppressed. During pregnancy, corpus luteum persists with the result that heat is not expressed and the cyclical activity is kept in abeyance. If pregnancy corpus luteum is either removed manually or regresses naturally, the cycles may recur with fear of abortion terminating the pregnancy.

Anoestrus is one of the symptoms amongst many conditions affecting the

course of the normal oestrous cycle. Error is likely to occur in case close watch is not kept on individual cows in the herd. In the absence of proper observations, a cow on heat may be missed and wrong diagnosis made that she is showing anoestrus, while rectal palpation will reveal the presence of corpus luteum indicating that she has expressed heat. In most cows anoestrous condition may be observed few months after parturition (post-partum) or it may follow service to which there has been no conception (post-service).

In heifers, it may pose as a herd problem (puberal anoestrus) possibly due to low plane of nutrition and stress of seasonal transition or extremes of climatic conditions and due to heavy lactation (lactational anoestrus). Failure of oestrus may occur due to multiple causes.

### True anoestrous condition

In this condition, there is insufficient pituitary stimulus for liberation of FSH and LH in consequence of which there is no maturation and rupture of the Graafian follicles in good time with liberation of viable ova. On palpation per rectum it will be found that both

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tion in early pregnancies. In case one is not sure of pregnancy, it is better to carry out repeat examinations to find if there is progressive development specially in animals with history of service and where bull is running free with the cows.

#### **Anoestrus due to retained or persistent corpus luteum**

Persistent corpus luteum is mostly associated with pathological conditions of the uterus such as pyometra, mummification and maceration. The condition needs repeated examinations for confirmation.

#### *Retained corpus luteum associated with mummification, pyometra and maceration of the foetus :*

In cases where these conditions exist for months or even longer, the corpus luteum becomes centrally located in the ovary, is hard and difficult to palpate. In such cases, it is difficult to remove the deeply embedded or retained corpus luteum and enucleation may damage the ovary. Breeding history associated with actual examination of the type of corpus luteum present is helpful in diagnosis of these conditions.

nic deaths occurring before 90 days, the embryo or foetus is very small and abortions may go unnoticed, or the foetus gets macerated in the uterus which may cause delay in the on-set of the oestrus, until such a time of complete resorption. After the embryo or foetus is expelled or resorbed, the cow usually returns to normal oestrus. The condition has been observed in Brucellosis, Vibriosis and Trichomoniasis. Imperfectly developed zygotes due to defective sperm or ova, may perish within 15 to 90 days after conception and produce a similar condition of delayed oestrus due to retained corpus luteum. Various reports show a marked difference between the percentage of cows actually pregnant and the percentage of cows apparently pregnant based on non-return basis at 28 to 35 days or 60 to 90 days after service. Apparently this discrepancy is due to early embryonic death.

It is commonly observed that even after normal service many cows fail to conceive and subsequently they may come back to oestrus after a period which is usually longer than the normal cycle. In a number of instances this may be due to early death of the embryo and this delayed onset of oestrus or period of anoestrus after the loss



ovaries are usually small, smooth and round. In certain cases follicles may develop upto prematuration size, in which case the ovaries may become rounded. Fresh corpora lutea are not present. In heifers, ovaries are usually smooth. In anoestrus cow, the old corpora lutea may roughen the ovarian surface. The absence of corpus luteum distinguishes anoestrus condition from both the diestrus and early pregnancy. In anoestrus condition, the uterus is invariably flaccid, whereas it has good tone and become turgid during oestrus. Difficulty may arise in distinguishing anoestrus condition from early period of diestrus, during late stages of oestrous cycle, close to proestrus period when the corpus luteum is regressing. A second examination after 10 to 12 days may prove beneficial in the differential diagnosis. In dairy cows in great Britain, anoestrus condition is commonly observed and is a great problem. It is chiefly a winter affection and it resolves spontaneously in spring. The farmers expect that the majority of dairy heifers should calve in the autumn and this entails breeding during winter. It is difficult to feed heifers during winter months, which may affect their reproductive capacity. In these circumstances, it is likely that heifers that were having normal cycles in the autumn may pass into anoestrus condition in winter and the cycles may not be resumed until the following spring or early summer. The endocrine system of heifers exposed to decreasing day light intensity are very susceptible to dietary deficiency, of which the most important is protein. The pituitary gonadotropic output soon becomes most insufficient.

Chronic debilitating diseases such as intestinal worms or liver fluke infestation may interfere with assimilation of

food and will tend to cause anoestrus condition.

Deficiency of any dietary constituent which is necessary for normal metabolism such as iron, copper, cobalt, manganese, iodine, phosphorus and vitamin A is a probable cause of unthriftiness condition which may give rise to anoestrus. In Great Britain, deficiency of copper is the most likely dietary cause of anoestrus condition. Certain areas are deficient in iodine manifesting congenital goitre in calves resulting in anoestrus condition.

Post parturient cows are the most likely ones exhibiting anoestrus condition particularly in cows that have calved in late autumn or winter, with the result that the first obvious heat of such cows may not be seen until the following spring. Suckling dams, first and second calvers, high lactation, insufficient feeds and extremes of weather may prolong the postparturient anoestrus. It is therefore advisable to feed dairy cows well before they calve as well as immediately after parturition and then onwards until the lactation is completed. Good animal husbandry practices can prevent occurrence of anoestrus condition.

### Anoestrus due to pregnancy

Certain number of cows which show oestrus during pregnancy, give a false impression that they are not pregnant. It is therefore advisable to examine the genital tract by palpation per rectum as a routine during periodic check up to ascertain, if the cow is pregnant. It is dangerous to enucleate corpus luteum just relying on history only. Instances are on record that when by mistake, corpus luteum has been removed to induce oestrus in cows, it has led to abor-



Fig. 72. Bilateral hydrosalpinx with right cystic ovary in a buffalo

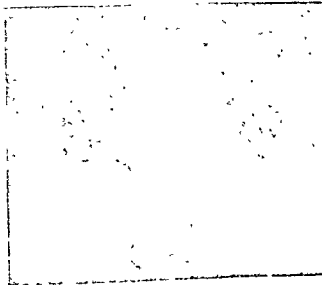


Fig. 73. Bilateral hydrosalpinx in a buffalo

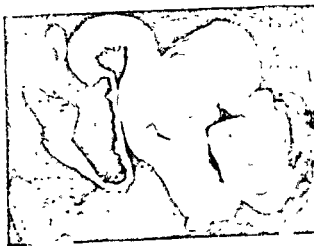


Fig. 74. Unilateral hydrosalpinx in a buffalo.



the presence of periodical corpus luteum indicating that heat followed by ovulation has occurred. Occasionally silent heat may follow a normal heat after calving.

Failure to exhibit the typical symptoms of heat may be due to lack of secretion of oestradiol by the mature follicles, or it may be due to a need for a higher threshold for oestrogen in certain individuals to produce the typical symptoms characteristic of oestrus and consequent production of intense desire for acceptance of the bull. However, quality of oestrus is dependent upon estrogen progesterone ratio. Lagerlof (1957) postulated that certain strains in cattle have a hereditary predisposition for weak oestrus. According to Vandeplassche (1972) inadequate sensitization by progesterone is probably a cause of silent heat.

Silent oestrus or suboestrus occurs more frequently between calving and 60 days post-partum. Kidder *et al.* (1952) reported on 286 ovulations of which 78 or (27.3%) followed silent heat. The incidence of silent heat during the first 60 days of calving was 44.3% of 140 ovulations. But there were 11% in 146 ovulations between 60 to 308 days. Casida and Wisnicky (1950) reported that in majority of cows ovulations occurred at an average of 25.4 days earlier than the first post-partum oestrus and the 68% of the cows showed at least one silent heat before the first clinically apparent oestrus. Cows older than 5 years tended to show longer intervals between calving and the first heat as compared to the younger ones. Trimberger (1955) noted that about 10% of the ovulations during the service period were silent. Thus it is evident that silent heat is probably

the most common cause of sub-oestrus which considerably delays rebreeding of animals. The condition is clinically characterised by failure of oestrus even though the animals are ovulating.

### Unobserved Oestrus

In this condition, the oestrous periods are usually short in length and particularly in heifers the periods may be as short as that of 6-8 hours. In case, the cows are not turned out morning and evening, silent heat cases may be missed and herds in which there is such a large number of weak or silent oestrus may go difficult for the farmer to detect and greatest difficulty is usually experienced if they are to be reported for breeding by artificial insemination. In such cases it is better to run a bull with the herd. Vasectomised bulls may be maintained to detect heats in case the cows are to be bred by artificial insemination. Olds and Seath (1954) recorded that about 30% of the prolonged oestrous cycles were due to missed heat periods. Zemjanis (1961) reported anoestrus as 39% of 5848 reproductive cycles in dairy cows. He further stated that it was 77% in pre-service cycles and 28% in post service cycles.

### Anoestrus due to cystic corpora lutea

The incidence of cystic corpora lutea is very small as that of 3% only, of the cases of cystic ovary. In such a condition a spherical, smooth, tense structure measuring about 2.5 to 5 cm. in diameter is felt on rectal palpation (Roberts, 1956). The feel is like a thick cystic follicle. Cystic corpus luteum is usually a single structure whereas cystic ovarian follicles may be single or multiple. Compared to follicular

cyst, luteal cyst is firmer and much less fluctuating. However, fluid in the central cavity can be palpated. On manual pressure, the fluid may burst out causing collapse of the shell of the lutein tissue. Wright (1915) concluded that about 25% of all corpora lutea have a small non-pathogenic central cavity measuring about 0.4 to 0.6 cm in diameter. The normal corpora lutea are irregular in shape indicating that ovulation has occurred. The cystic corpora lutea are in most cases spherical indicating that ovulation has not occurred. In this condition a recognisable amount of lutein tissue is found lining the wall of the cysts. The presence of the lutein tissue prevents the occurrence of the oestrus and produces symptoms of prolonged anoestrus. Trimberger and Hansel (1954) reported that cystic corpus luteum is a result of endocrine upset. A normal pregnancy has not been observed in the presence of a true cystic corpus luteum.

Manual removal of the cystic corpus luteum may cause severe bleeding with the consequent development of adhesions that may interfere with future conceptions.

#### **Anoestrus secondary to debility or marked loss in weight**

Malnutrition for prolonged periods will have an adverse effect on the functioning of the reproductive system which usually causes anoestrus. Debilitating condition may suppress the occurrence of estrous cycles and produce anoestrus. Some such conditions are mentioned below: (a) A very low plane of nutrition due to lack of protein, carbo-hydrates and fat may cause delay or delay in the onset of the

puberty. This condition is often observed in yearling heifers maintained on poor hay and a minimum amount of concentrates during the winter months. Deficiency of proteins, carbohydrates, micro-macro elements and vitamins may lead to anorexia and debility with impairment or suppression in the liberation of gonadotropic hormones. In rats, a loss of 15% in body weight may cause cessation of the oestrous cycle. In human, starvation causes amenorrhoea and cessation of menstrual cycle, before other disturbances become apparent. Even deficiency of sodium chloride for prolonged periods may affect oestrous cycle and the effects become very apparent after parturition, in farm animals. In such cases, the lactational stress causes rapid loss of weight since the appetite is poor. Milk secretion declines and may even cease. The cows having become cachectic, fail to show oestrus. On rectal palpation the ovaries are found to be small and inactive. Animals may not return to oestrus until sodium chloride is supplied and the appetite, plane of nutrition and health of the animals improve. Sane *et al* (1967) reported anoestrous condition in Dangi cattle, fed on poor plane of nutrition during winter months. Similar condition may also be observed during severe dry summer periods in several parts of India particularly on the coastal sides, where available feed greatly becomes scarce during summer season. Rapid loss in body weight may result due to heavy lactation, and if it is extreme it may cause cessation of oestrous cycle. If the loss in body weight after calving is gradual, one or two heats may be exhibited before anoestrus develops. In lactating cows, which are showing anoestrus due to malnutrition, production of milk is usually, low. In the event of low nutri-

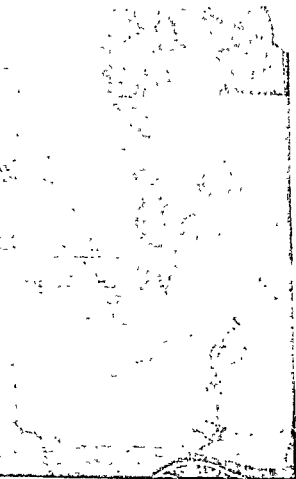


Fig. 78. Perimetritis with extensive adhesions with the bladder in a buffalo.

← Fig. 77. Mucometra in a cow.

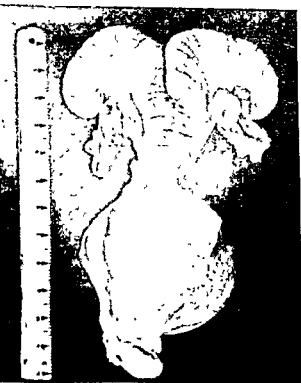


Fig. 79. Perimetritis with surface veins showing varicosity in a buffalo.

← Fig. 80. Abscess in the vagina of a buffalo.

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tive intake by large number of cows on the same farm, anoestrus will occur in majority of heifers and cows. Rectal palpation in such cases will show small inactive ovaries. There is no palpable corpus luteum and follicles. In most cases, the uterus is atonic, and there is flaccid feel. Vaginal examination will reveal pale and dry condition of the mucous membrane and the cervix tightly closed. The affected animals usually appear thin and have rough coats and dry faeces.

### Chronic or debilitating diseases

These usually cause decreased appetite which may result in failure of oestrus due to marked reduction in bodyweight. Severe mange, severe chronic acetonæmia, lymphocytoma, displacement of abomasum, chronic traumatic gastritis, chronic pneumonia, Johnes disease, severe internal or external parasitism, suppurative arthritis, footrot, advanced tuberculosis and similar other conditions may result in debility with consequent failure of oestrus. Vitamin A deficiency leads to hyperkeratosis, marked metaplasia of the cervix and endometrium as well as skin and digestive lesions, debility and anoestrus.

### Old age or senility

Old age may bring about debility, leading to the failure of reproductive functions but menopausal syndrome encountered in human is not present in cows and buffaloes.

### Prognosis of anaestrous condition

Prognosis of anoestrus in cows and buffaloes depends upon the causative factors.

## Treatment

Treatment in the case of failure of oestrus will vary from case to case depending on the causative factors. If failure of oestrus is due to hereditary factors, treatment is contraindicated. Treatment should only be undertaken if anoestrus is due to pathological conditions of the reproductive tract or due to low level of nutrition. Results of hormone therapy are most uncertain.

## Miscellaneous conditions

### NON FUNCTIONAL OR SMOOTH OVARIES

This condition may occasionally be observed in well fed cattle in good condition during the first two to four months after calving and during the high phase of lactation. Buch *et al* (1955) reported that cows milked three times a day and producing heavy amounts of milk failed to show first oestrus after parturition for months. The oestrus in these cows occurred at an average of 60 days after calving as compared with an average of 47 days in all cows. Trimberger (1955) reported in his observations that 7.5% of the cows and 3.9% of the oestrous cycles studied were characterised by smooth ovaries with varying periods of anoestrus. Recovery in such cases may occur spontaneously.

### MULTIPLE OVULATION

Kidder *et al* (1952) reported on 294 ovulations which included 252 single and 42 multiple ovulations as diagnosed by rectal palpation, the incidence of multiple ovulation was 14.3%. In single ovulations the conception rate was 57.5% and in multiple ovulations it was 28.6%.

In cases of delayed ovulations, synchronization of ovulation and service

may not occur in majority of cases resulting in transient form of infertility. Suspected delayed ovulation or failure of ovulation may be indicated by slightly prolonged oestrous cycle and palpation of mature follicle on the ovary even after 36 to 48 hours after the cessation of oestrus (Hanbrich, 1955).

#### FREEMARTINISM

The condition is rare. On rectal examination ovaries and uterus usually cannot be palpated except as thin remnants of the normal structures. Vagina is underdeveloped. Such animals are sterile.

#### CONGENITAL BILATERAL HYPOPLASIA OF THE OVARIES

This is rarely observed. Fincher (1946) reported complete absence of gonads in several related cattle. In rare cases heifers may have a fairly normal genital tract but very small flattened hypoplastic ovaries of the size of a bean or even smaller, 0.6 cm. long and 0.3 cm. in diameter. Either one or both ovaries may be hypoplastic, small and inactive. (Fig. 71). Especially in buffalo heifers such small size of normal ovaries may erroneously be diagnosed as hypoplastic ovaries (Sane *et al*, 1961). Heifers should be allowed to reach full growth before making final diagnosis of congenital ovarian hypoplasia. Complete bilateral hypoplasia results in sterility.

#### TUMOURS OF THE OVARY

These are rare and hardly account for failure of oestrus. The most common form in the cow is the granulosa cell tumour, which may cause anoestrus or occasional symptoms of nymphomania. However, the fact that the granulosa cell tumours are found in related cattle, should not be overlooked if the cows are to be used for further breeding.

#### CYSTIC OVARIES

These may be characterised by failure of oestrus in about 25% of cases. One or more of the follicular cysts can be palpated per rectum. In this condition the uterus is usually doughy and flaccid. The vaginal mucus may be tenacious. There may be oedema of the vulva in varying degrees and relaxation of pelvic ligaments. Few cases of mucometra are associated with cystic condition of the ovaries and failure of oestrus for months. Many a time the condition may be confused for pregnancy. Repeated examinations are therefore necessary in case of doubts. In such a condition failure of oestrus characterised by hypertrophied and dilated cervix, filled with copious amounts of tenacious tough mucus, resembling the mucous of the cervical seal during pregnancy is usually observed (Roberts 1956).

#### Ovaritis

This may occur due to infection, trauma, frequent massage or rough palpation of ovaries per rectum, undue pressure exerted during enucleation of corpus luteum or rupture of cyst. Adhesions may develop due to inflammatory condition caused by ascending or descending infection. Paraovarian cysts may be found in the broad ligaments of the cow or buffalo around the ovaries and ovi-ducts. Paraovarian cysts are more commonly observed in the mare, ewe and bitch.

#### Affections of the oviduct

The etiological factors causing pathological conditions of the oviducts may be ascending or descending type of infections following peritonitis and due to conditions such as abortion, retained placenta, septic metritis and pyometra. Perimetritis may cause extensive adhe-



sions of the oviduct, ovary and uterus to the adjacent structures.

Animals with salpingitis may become infertile or sterile depending on the severity of the condition. Inflammatory condition, whether acute or chronic may lead to the thickening of the mucosal rugae because of oedema in the substantia propria, congestion of blood vessels and the leucocytic infiltration in acute inflammation and varying degree of proliferation of fibroblastic connective tissue cells especially in lamina propria in chronic inflammation. Moreover bursal adhesions, cysts over the oviducts and adhesions of pavillion with ovary may obliterate lumen of the oviducts, thus obstructing the passage of ovum resulting in infertility or sterility.

Infective organisms such as Streptococci, Staphylococci, *E. coli*, *C. pyogenes* have been detected by various authors. Many authors have observed that the treatment for infertility or sterility has become a major factor in inducing inflammatory conditions of the oviducts and bursa. Moberg (1954) observed that haemorrhage occurring due to enucleation of corpus luteum and haemorrhage occurring at ovulation and displacement of corpus luteum in the ovarian bursa may give rise to bursal adhesions. Similarly manual rupture of thick walled follicular cysts or cystic corpora lutea may cause adhesions. Uterine irrigations with antiseptic solutions may escape in oviducts resulting in inflammatory condition of oviducts.

The incidence of pathological changes reported in cows from the abattoir material was — Carpenter *et al* (1921) 15.3%, Rowson (1912) 13.0%, Spriggs (1915) 4%, Cembrowicz (1950), 8.9%, McEntee (1954) 10%, Moberg (1954) 15.5% and Mylrea (1961) 3.6%, Salpingitis and 9.9% bursitis. The incidence

as reported in buffaloes from the abattoir material was Shalash (1958) 51% and Deshpande and Sane (1967 & 1971) 22.31%. Calaprice (1961) observed salpingitis in buffaloes due to *B. abortus* associated with *E. coli* and in few he observed Streptococci, Staphylococci and Tuberculous infection.

### Hydrosalpinx

Hydrosalpinx may occur secondary to segmental aplasia of the paramesonephric duct and other anomalies of the reproductive tract. Adhesions at the proximal or distal ends of the oviducts may cause hydrosalpinx. Hydrosalpinx was clinically diagnosed in 8 unbred buffalo heifers (Anon, 1962). (Fig. 72, 73, 74).

### Pyosalpinx

It usually occurs as a result of severe infection in the uterus. It is associated with adhesions of the mesosalpinx and meso-ovarium. (Fig. 75). Perimetritis is not uncommon. Repeated use of Stilboesterol for the treatment of pyometra may give rise to pyosalpinx. Abscess or cyst formation is likely to occur between the bursa and the ovary and the fimbrial end becomes attached round the edges of the ovary.

The diagnosis of affections of oviduct in mild cases is rather difficult by rectal palpations. However in severe cases with adhesions including ovary, oviduct and surrounding structures, the diagnosis is easy. Cases of perimetritis, ovaritis, hydrosalpinx, pyosalpinx and abscesses can be diagnosed by rectal palpations. Repeat breeding may give suspicion of the inflammatory conditions of the oviduct.

Peritoneoscopy and visualization of the ovaries and oviduct in cows and

buffaloes and also Rubin insufflation apparatus have very limited scope in the diagnosis.

Prognosis is usually unfavourable except in transient forms of mild infections.

### Treatment

Salpingitis invariably co-exists with metritis, in consequence of which treatment for both conditions will be the same. Prevention is more important than the treatment. Parenteral as well as intrauterine medication of antibiotics may prove beneficial, provided there is no blockage of oviducts or damage due to adhesions at the fimbriated extremities.

Pushing large amounts of antiseptic fluids for cleansing the uterus should be avoided.

### Seasonal influence

Intensity of sunlight, variations in temperature, humidity and exercise has influence on oestrous cycle and oestrus. However the effect is not so pronounced as that of nutrition. Failure of oestrus has often been observed in cows and heifers confined in dark stables for prolonged periods. Mercier and Salisbury (1947) have shown that even though the cow is a polyoestrous animal, the best conception rates occurred during summer months, and poorest during winter in the northern part of the country where cows are mostly kept indoors during winter months. This is indicative of the fact that in addition to the feeding level, the type of feed, amount of exercise, intensity and amount of sunlight available and its effect on the pituitary has considerable impact on the occurrence of oestrus.

### Infertility due to Nutritional causes

Most cases of reduced fertility or infertility are usually due to multiple deficiencies. In a few cases only, nutrients have a direct effect on fertility. Underfeeding may be accompanied by poor quality of feed and by deficiencies of proteins, phosphorus and vitamin A. Protein deficiency is usually accompanied by phosphorus deficiency, and vitamin 'A' deficiency accompanied by protein and phosphorus deficiency. Many a times reduced fertility believed to be of nutritional origin may be the effect of adverse environmental conditions. Symptoms of deficiency may vary from species to species. Young animals are more susceptible to nutritional deficiencies which has consequent effect upon growth, puberty and sexual maturity. Lactation causes heavy drain, especially on minerals.

### Underfeeding, inanition or starvation

Underfeeding delays sexual maturity in heifers and may cause inhibition of oestrous cycle in all mammals. In adult animals underfeeding for prolonged periods or starvation may cause failure of follicular development to maturity resulting in follicular atresia. Feeding in restricted quantities may have an effect on the early embryo. But starvation to a certain extent after this period does not necessarily interrupt gestation but may cause birth of small, weak young ones or still births. In older cattle anoestrus or irregular oestrus periods are usually manifested as a result of underfeeding.

### Obesity

Overfeeding may

observed in beef breeds in which over feeding to fatten the animals in a short time is a general practice. In these animals the ovaries are small with few large follicles. Oestrous cycles may become irregular or may fail to occur. Deposition of fat in the ovarian bursa and ovaries may interfere with normal ovulation and transport of ovum to the pavilion of oviduct. Obesity and sterility might occur due to hypothyroidism or lesion in the pituitary. Hansel (1953) reported that in a controlled experiment where animals were kept on a high plane of nutrition throughout their life resulted in a very few calvings and the group had a higher percentage of culling due to infertility than the ones fed on normal or low plane of nutrition. Reports from New Zealand and Cornell and the Swedish data indicate that heavy feeding hastens the onset of puberty but may result in slight increase in the number of services per conception and may lower milk yields and also cause shorter life.

### Protein deficiency

Deficiency of protein either qualitatively or quantitatively is rarely observed. Deficiency is seldom encountered except due to underfeeding or in severe inanition where Vitamin A and phosphorus deficiencies are also the complicating factors. In ruminants however sufficient protein is synthesized to support reproductive functions if the level of protein in the general feeding is fairly adequate.

### Carbohydrate deficiency

Lack of carbohydrates may cause inanition and loss of body weight with consequent effect on fertility.

### FAT DEFICIENCY

The fat deficiency is not probably encountered in ruminants. Their feed contains fairly good amount of fat. Other nutrients can also be converted into fat.

### Vitamin deficiencies

In cattle Vitamin 'A' has considerable effect on fertility. Vitamin 'A' deficiency adversely affects reproduction in most of the species. The effects are markedly observed during the latter half of gestation and are characterised by abortion or birth of weak or dead calves. It is remarkable that Vitamin 'A' deficient animals may have normal oestrous cycles, ovulation and conception and early foetal development occurs even though epithelial and other tissue changes have developed. Vitamin 'A' requirements are higher during pregnancy. Vitamin 'A' deficiency is characterised by changes in the epithelial tissue such as Keratinization and degeneration of the placenta. Dystokia, retained placenta and metritis are likely to result. Vitamin 'A' deficiency may occur in cattle grazing on dry bleached pastures and grain fields where there was possible deficiency of protein, phosphorus and carbohydrate. In a group of such animals when grain feeding was supplemented, night blindness, Ichthyomatia, anorexia, diarrhoea, loss of condition and other symptoms of Vitamin 'A' deficiency as well as abortion and still birth ceased to occur. Symptoms of Vitamin 'A' deficiency take months to develop since the liver has the ability to store considerable amounts of Vitamin 'A'.

### Vitamin 'B' Complex deficiencies

Deficiency of Vitamin 'B' may produce inhibitory effects on reproduction.

Deficiencies of Vitamin 'B' are usually accompanied by reduced appetite. Animal on low Vitamin 'B' complex levels may show delayed sexual maturity and atrophy of the ovaries and uterus. Deficiency of Vitamin 'B' may affect the secretion of gonadotropic hormones. Liver may fail to inactivate oestrogens due to low levels of Vitamin 'B'. On account of the ruminal synthesis of B complex Vitamins, there is no fear of the occurrence of Vitamin 'B' deficiencies of cattle. Vitamin B12 requires Cobalt for its synthesis. In cobalt deficient areas, cattle may develop severe inanition due to lack of sufficient intake of feed containing cobalt.

#### Vitamin 'C' deficiency

Deficiency of Vitamin 'C' does not occur in cattle. It is only seen in man, monkeys and guinea pigs. But even in these species there is no reproductive failure as a result of deficiency of Vitamin 'C'.

#### Vitamin 'D' deficiency

It is available in plenty in roughages, greens and through sunlight. Rid (1949) reported that few cows fed on a very low Vitamin 'D' intake failed to come into oestrus.

#### Vitamin 'E' deficiency

Oestrus and oestrous cycles may occur normally, in spite of Vitamin 'E' deficiency. In rats the deficiency may interrupt pregnancy after midterm by resorption of foetuses. In the male rat Vitamin 'E' deficiency causes irreparable damage to the epithelium of the seminiferous tubules. In herbivorous animals liberal quantities are always made available in the natural feed and cows can reproduce even with some deficiency. Salisbury (1944) has shown

that additional supplement of Vitamin 'E' fed to bulls had no effect on fertility. Gullickson *et al* (1949) observed that bulls and heifers even though fed on Vitamin 'E' deficient feeds, were fertile. Feeding wheat germ oil does not appear to show significant differences between treated and controlled groups of cattle.

#### Mineral deficiencies

The deficiency causing infertility in cattle are limited to phosphorus and occasionally to trace elements. Phosphorus deficiency usually occurs when feeds low in protein are fed under range conditions when the grass becomes dry and in parts where the soil is also deficient in phosphorus. Lactating cows fed poorly on proteins and mineral supplements fail to maintain the necessary body levels of phosphorus. Reproduction usually does not suffer until such a time when the clinical symptoms such as inanition, poor haircoat and depraved appetite become evident. In the heifers ovulation may occur without clinical symptoms of oestrus. The usual symptoms of phosphorus deficiency are delayed onset of puberty in heifers and failure of oestrus in cows. However if cows come in oestrus, conception and gestation is not interfered with unless complicated with protein and Vitamin A deficiency. In some cases the calves may be weak or stillborn, but abortions do not occur.

Calcium deficiency normally should not cause failure of reproduction in cattle.

Manganese deficiency may cause deaths of the new born in rats. In the male rats it is also necessary for the maintenance of the seminiferous epithelium. There are no reports of manganese deficiency in cattle.

Cobalt deficiency may cause depraved appetite, inanition, failure of oestrus and delayed onset of puberty in cattle. Cobalt is concerned in the production of Vitamin B<sub>12</sub> in ruminants. In cobalt deficient cows, weak or dead foetuses may be expelled at term.

Copper and Iron deficiency may cause lack of appetite, anaemia and debility. It may cause secondary inanition and delayed puberty. A high molybdenum content in the feed may result in severe scouring and loss of condition, particularly when copper is not supplemented in the feed. The loss of condition may be reflected in failure of oestrus in cows or in delayed onset of puberty, in heifers. Wallace (1949) reported that copper deficient cattle in New Zealand have marked general effects on reproduction.

Iodine deficiency in the northwest part of United States caused birth of weak, premature and dead calves affected with goitre. Lack of iodine can depress thyroid function and indirectly affect fertility. Hyper thyroidism or hypothyroidism may reduce the secretion of gonadotropic hormones by the anterior pituitary. There is no evidence to show that iodine deficiency either affects the oestrous cycle or conception.

Mineral supplement may be necessary for lactating cows. Mokashi *et al* (1974) demonstrated the beneficial effect on body weight changes, incidence of oestrus and conception in a group of anoestrus Gir cows by increasing digestible energy from 14.0 megacal, to 20.5 megacal. Estimation of blood cholesterol, total serum protein, blood glucose and body weight have been found to be useful parameters in identifying the infertility problem as well as to carry out suitable remedial measures (Deopurkar

1974 and Patil 1976). Deshpande and Sane (1977) and Deshpande (1977) have reviewed various therapies viz. oral administration of herbal indigenous drugs like Prajana, Heatrone, Aloes compound and non-hormonal drug Fervivet to combat bovine infertility.

A very large proportion of conception failures can be attributed to varying degrees of endometritis, chronic metritis and salpingitis.

### Endometritis

Endometritis is a very common cause of infertility in cows and buffaloes which is due to inflammatory condition of uterus on account of bacterial, viral or fungal infections.

### Aetiology

In great majority of the cases, metritis is due to infection, the severity of inflammation being related to the virulence of organisms involved. However, a variety of factors predispose the uterus to infection and these include wounds on uterine mucosa, dystokia, abnormal parturition, abortions, retained afterbirths, twin births, premature births, uterine inertia, lack of exercise, delayed involution, crude methods of treatment, injury during insemination and unhygienic conditions at the time of calving. In addition to these the nutritional deficiencies, unbalanced rations such as excess of potassium in fodder and exposure to wet and cold may lower the resistance and predispose to infections. Hormonal imbalance such as excess of oestrogens may give rise to cystic form of endometritis (Van Rensberg, 1957). Hignett (1952) attributed thyroid insufficiency or iodine deficiency as a predisposing factor for endometritis. Lagerlof (1918) and Koch *et al* (1958)

tional stress. Endometritis is occasionally observed in cows or buffaloes which have pneumovagina. The air, faeces and urine may be drawn into the ballooned vagina leading to chronic septic metritis, seen particularly in older cows. Metritis in cows has been reported to occur following coitus due to the transmission of organisms from the sheath of bull or through semen. The natural defences against infection during oestrus help the cow to overcome this massive infection.

Many saprophytes exist in the bovine genital tract. Of the specimens studied by Dawson (1959), 20 to 40% contained bacteria commonest of which were *Streptococci*, *Micrococci* and *E. coli*. *Neisseria*, *Pseudomonas*, *Proteus*, *C. viridans* were few. They may act as secondary invaders subsequent to infection by specific uterine pathogens. This is called as "facultative pathogenic infertility".

However, a large number of organisms have been reported to be associated with bovine endometritis. According to Dawson (1960) there is general agreement that those most commonly encountered are *Staphylococci*, *E. coli* and *Haemolytic Streptococci*, *Proteus*, *Pseudomonas pyogenes*, and *Neisseria* occur rather consistently as minority organisms. He opined that 15 — 40% of endometritis cases were due to *C. pyogenes*.

[*Pleuropneumonia* like organisms, moulds and fungi were also incriminated as responsible for metritis (Kiesel and Dacies, 1939). Miller (1935) reported a filtrable virus capable of inducing endometritis in cattle.]

endometritis have been reported following workers — M. tube in buffaloes (Polding and Lall Sane *et al*, 1959, Deshpande *et al* and Bhandari *et al* 1968). (Fig. foetus (Ray *et al* 1956). V. (Mohan, 1954, Parnaik *et al*, Amritkar (1973) recorded in 18 metritis in a herd of Gir cows in presence of following organisms — of *staphylococci*, *streptococci*, *E. subtilis*, *Proteus* and *Klebsiella*.

### Incidence

The incidence of endometritis in cows varies greatly. Parson-Son (1959) reported the incidence as 9.2%. Anand (1973) reported the incidence of metritis as 11.5% based on actual findings in a herd of Gir cows.

In buffaloes, the incidence has been reported as 27.15% (Bhattacharya 1954), 21.74% (Luktuke, 1958), 11.3% (Malik 1960) and 24.52% (Velhankar Purohit, 1965-66).

### Symptoms

Clinical symptoms of endometritis cannot be demonstrated by vaginal examinations or rectal palpation of the uterus, especially during oestrus. A mucopurulent discharge is observed in severe cases. Flakes of pus are present in the oestral discharge. Flakes may be from cervix or uterus both. In endometritis oestral discharge is often cloudy or milky instead of being clear and translucent. A mild severe cervicitis may be present in many cases associated with endometritis.

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Copper and Iron deficiency may cause lack of appetite anaemia and debility It may cause secondary inanition and delayed puberty A high molybdenum content in the feed may result in severe scouring and loss of condition particularly when copper is not supplemented in the feed The loss of condition may be reflected in failure of oestrus in cows or in delayed onset of puberty in heifers Wallace (1949) reported that copper deficient cattle in New Zealand have marked general effects on reproduction

Iodine deficiency in the northwest part of United States caused birth of weak premature and dead calves affected with goitre Lack of iodine can depress thyroid function and indirectly affect fertility Hyper thyroidism or hypothyroidism may reduce the secretion of gonadotropic hormones by the anterior pituitary There is no evidence to show that iodine deficiency either affects the oestrous cycle or conception

Mineral supplement may be necessary for lactating cows Mokashi *et al* (1971) demonstrated the beneficial effect on body weight changes incidence of oestrus and conception in a group of anoestrus Gir cows by increasing digestible energy from 110 megacal to 205 megacal Estimation of blood cholesterol total serum protein blood glucose and body weight have been found to be useful parameters in identifying the infertility problem as well as to carry out suitable remedial measures (Deopurkar

1974 and Patil 1976) Deshpande and Sine (1977) and Deshpande (1977) have reviewed various therapies viz oral administration of herbal indigenous drugs like Prajana Heatrone Aloes compound and non hormonal drug Fervivet to combat bovine infertility

A very large proportion of conception failures can be attributed to varying degrees of endometritis chronic metritis and salpingitis

### Endometritis

Endometritis is a very common cause of infertility in cows and buffaloes which is due to inflammatory condition of uterus on account of bacterial viral or fungal infections

### Aetiology

In great majority of the cases metritis is due to infection the severity of inflammation being related to the virulence of organisms involved However a variety of factors predispose the uterus to infection and these include wounds on uterine mucosa dystokia abnormal parturition abortions retained after births twin births premature births uterine inertia lack of exercise delayed involution crude methods of treatment injury during insemination and unhygienic conditions at the time of calving In addition to these the nutritional deficiencies unbalanced rations such as excess of potassium in fodder and exposure to wet and cold may lower the resistance and predispose to infections Hormonal imbalance such as excess of oestrogens may give rise to cystic form of endometritis (Van Rensberg 1957) Hignett (1952) attributed thyroid insufficiency or iodine deficiency as a predisposing factor for endometritis Lagerlof (1918) and Koch *et al* (1958)

considered that hereditary tendency in animals of weak endocrine constitution as predisposing factor, whereas, Renner (1956) considered it to be due to lactational stress. Endometritis is occasionally observed in cows or buffaloes which have pneumovagina. The air, faeces and urine may be drawn into the ballooned vagina leading to chronic septic metritis, seen particularly in older cows. Metritis in cows has been reported to occur following coitus due to the transmission of organisms from the sheath of bull or through semen. The natural defences against infection during oestrus help the cow to overcome this massive infection.

Many saprophytes exist in the bovine genital tract. Of the specimens studied by Dawson (1959), 20 to 40% contained bacteria commonest of which were Streptococci, Micrococci and *E. coli*. Neisseria, Pseudomonas, Proteus, *C. viridans* were few. They may act as secondary invaders subsequent to infection by specific uterine pathogens. This is called as "facultative pathogenic infertility".

However, a large number of organisms have been reported to be associated with bovine endometritis. According to Dawson (1960) there is general agreement that those most commonly encountered are Staphylococci, *E. coli* and Haemolytic Streptococci, Proteus, Pseudomonas pyogenes, and Neisseria occur rather consistently as minority organisms. He opined that 15 — 40% of endometritis cases were due to *C. pyogenes*.

[Pleuropneumonia like organisms, moulds and fungi were also incriminated as responsible for metritis (Kiesel and Daries, 1959). Miller (1955) reported a filtrable virus capable of inducing endometritis in cattle.]

The more specific uterine pathogens include *B. abortus*, *V. foetus*, *T. foetus* and *M. tuberculosis*. However, various infective organisms causing endometritis have been reported by the following workers — *M. tuberculosis* in buffaloes (Polding and Lall, 1945, Sane *et al*, 1959, Deshpande *et al*, 1966 and Bhandari *et al* 1968). (*Fig. 1*). *T. foetus* (Ray *et al* 1956). *V. foetus* (Mohan, 1954, Parnaik *et al*, 1956). Amritkar (1973) recorded in 18 cases of metritis in a herd of Gir cows the presence of following organisms — species of staphylococci, streptococci, *E. coli*, *B. subtilis*, Proteus and Klebsiella.

### Incidence

The incidence of endometritis in cows varies greatly. Parson-Son (1962), reported the incidence as 9.2%. Amritkar (1973) reported the incidence of endometritis as 11.5% based on actual calvings in a herd of Gir cows.

In buffaloes, the incidence has been reported as 27.15% (Bhattacharya *et al*, 1954), 21.74% (Luktuke, 1958), 12.9% (Sane *et al*, 1959), 11.3% (Malik *et al*, 1960) and 24.52% (Velhankar and Purohit, 1965-66).

### Symptoms

Clinical symptoms of endometritis cannot be demonstrated by vaginal examinations or rectal palpation of genitalia. A mucopurulent discharge from the uterus, especially during oestrus, is observed in severe cases. Flakes of pus are present in the oestral discharge. Flakes may be from cervix or uterus or both. In endometritis oestral discharge is often cloudy or milky instead of being clear and translucent. A mild to severe cervicitis may be present in many cases associated with endometritis.



It is only from the purulent material present in the discharge, the degree of severity in endometritis can be ascertained to a certain extent. No other clinical manifestations are apparently visible. On rectal palpation, uterus may be felt slightly enlarged, heavy and thick-walled. These changes may be found in one or both horns. Delay in the normal involution of the uterus commonly accompanies infection causing endometritis. Examination per rectum is not always reliable. The oestrus and oestrous cycles are usually of normal length. On account of infection of the uterus and abnormal fertilized ovum may and the cow repeats to oestrus. However, the oestrous cycles may vary in some cases due to early death and maceration of embryo as in Trichomoniasis, Vibriosis and other infections. Repeat breeding and failure of conception are the most common symptoms of endometritis. On account of variability of clinical symptoms, no accurate diagnosis is possible during field work.

### Diagnosis

Metritis can be diagnosed tentatively on history and clinical symptoms presented by the animal. However, this has to be confirmed by bacteriological and histological examination (biopsy samples). The isolation of organisms and subjecting them to pathogenicity tests will reveal and confirm the causative factor. For this purpose, the cervico-vaginal swabs or discharge from uterus or vagina may be collected. Direct examination of pus smears subsequent to staining may also reveal the organisms.

Biopsy samples have a limited value for the fact that the lesions in endometritis are not well established yet. The histological picture may become com-

plicated by cyclical changes. However, the lesions vary depending upon the type of endometritis. Acute catarrhal endometritis is characterised by exudative changes in the stroma, mucoid degeneration, patchy necrosis, shrinkage and atrophy of mucosa in addition to oedema of interglandular connective tissue and myometrium, minimal fibroblastic proliferation, proliferation of the uterine glands and infiltration of stroma by polymorphs. Chronic endometritis is more common in which mucosa is thickened, oedematous and infiltrated by mononuclear cells, lymphocytes, plasma cells and eosinophils. Tubular glands are atrophic with cystic dilatation. Periglandular fibrosis, gland site mass lesions, thickening and hyalinisation of myometrial vessels is marked together with fibrosis of stroma. Beaver *et al* (1922) and Quinlan (1929) in cows and Velhankar *et al* (1965) in buffaloes observed the above changes in metritis cases. Cystic endometritis on the other hand is characterised by clustering of leucocytes and round cells both in and around gland cavities, appearance of glandular epithelium and general fibrosis of stroma. Cysts are confined to mucosa which vary in size. The lesions of metritis in Brucellosis, Vibriosis and Trichomoniasis are more or less of ulcerative necrotic in nature, whereas tuberculous endometritis presents the typical tubercular lesion *viz* caseation, calcification and acid-fast organisms in addition to fibrotic changes.

### Prognosis

Prognosis is fairly good in most cases of endometritis if treatment is done in good time. With each repeated oestrus and oestrous cycle, the natural body defences and the changes produced within the uterus and the genital tract by the

oestrogens, aid in the process of recovery. Rowson *et al* (1953) and Black *et al* (1953) showed that infection introduced in the normal uterus during oestrus was rapidly overcome and did not establish itself, but when infection was introduced during the metoestrus stage of the oestrous cycle, it readily became established resulting in pyometra.

### Treatment

Various procedures have been recommended for treatment of endometritis. It is difficult to prescribe one line of treatment. The object is firstly to overcome the infection and then to aid the regeneration of the endometrium by stimulation in varying degrees. Douching of the uterus should be avoided. Hydrogen peroxide solution in cases with thick discharge may prove beneficial. To overcome the infective condition, use of antibiotics is essential but one has to make proper choice in relation to causative organisms. Response to antibiotics is usually good. Aqueous solutions are considered to be more effective. It is however necessary to repeat the dose on 4 to 5 successive days and later on alternate days for a week or so. The oestral discharge during first oestrus occurring after the treatment will decide, if the uterus has become free of infection. In case there is purulent discharge or flakes, treatment shall have to be repeated. In acute or severe cases, it is advisable to administer antibiotics both parenterally and intra-uterine. Depending on the type of condition of uterus, oestrogenic compounds may be administered concurrently with antibiotic treatment. Beneficial results have been claimed by using Diethylstilboestrol in the dose varying from 10-20 mgm intra-muscularly. The dose may be repeated if necessary after about a

week or so. However, the possible effects of oestrogenic compounds on the mammary functions should be taken into consideration as it is usually experienced that milk yield drops perceptibly subsequent to the administration either parenterally or intrauterine, the fall is specially marked in buffaloes.

In the treatment of endometritis, mild stimulus to the endometrium is considered necessary to stimulate uterine activity. For this purpose most beneficial results have been obtained by administration of Lugol's solution 5% diluted 20 times in distilled water given intra uterine in the dose of 100 to 250 ml. preferably by Obels' apparatus after the initial treatment with antibiotics.

Smythe (1942) reported that infusing douching fluid in excess of 100 ml. into the non-pregnant uterus of a heifer or more than 150 ml. of fluid in a cow's uterus may cause rupture of the uterus or force the fluid in its tissues. Sane (1956) reported that as a consequence of douching with pressure the fluid escaped in the peritoneal cavity resulting in peritonitis and death in buffaloes.

If a cow is having chronic pneumovagina or pneumo uterus, it is necessary to scarify and suture the vulva in the similar way as described for the mare (Caslicks operation). At the same time uterus and vagina should be treated with antibiotics. When a cow shows couple of normal oestrous cycles she may be bred by artificial insemination. There are no cases on record of pneumovagina in Indian breeds of cattle. It is advisable to cull such animals.

### Prevention of endometritis

Hygienic conditions of the stables and care during parturition to prevent infections of the uterus, would help to

prevent endometritis. Great precautions are necessary in treating retention of placenta cases so as to prevent complications resulting in endometritis. Control of Brucellosis, Vibriosis and Trichomoniasis is most essential. If breeding is done by artificial insemination method, semen should be deposited in the cervix and not uterus. Care should be taken during vaginal examination and uterine treatment as not to introduce infection in various parts of the genitalia. As far as possible breeding of cows within 60 days after calving should be avoided.

### Delayed involution of uterus

Delayed involution may predispose the uterus to endometritis. Involution of uterus may be delayed on account of variety of infective and non-infective factors and the line of treatment varies accordingly.

### Pyometra

In cows and buffaloes this is characterised by the accumulation of pus or mucopurulent material in the uterus accompanied by retained corpus luteum resulting in failure of oestrus. Pyometra usually develops following dystokia and retards involution of the uterus in such conditions as abortion, premature birth, dystokia, twin calving, retained placenta and septic metritis. Spontaneous recovery is seldom possible in pyometra. The condition may arise after service from an infected bull and when it does it is usually associated with the death and maceration of embryo and its membranes. The pus should invariably be examined to confirm that there is no Trichomoniasis. Infected insemination pipettes and catheters may spread the infection to the uterus. Some cows may show oestrus during

gestation and it is risky to inseminate such cows as it may result in abortion and pyometra. Infection due to infected semen may cause death of embryo, its maceration and pyometra. In rare instances, pyometra may develop secondary to mucometra in white heifers Disease or in cystic condition of the ovary.

### Incidence

The incidence of pyometra in buffaloes has been reported as 2.47% (Shalash, 1958), 3.4% (Malik *et al*, 1960) and 3.92% (Velhankar and Purohit, 1966). The quantity of pus varies from 1.5 to 5 litres (Polding and Lall, 1915; Shalash, 1958 and Velhankar and Purohit, 1966).

### Symptoms

Failure of oestrus and retention of pus are the characteristic symptoms. The amount of pus may vary from  $\frac{1}{4}$  litre to 10-20 litres. In most cases the cervix will be found relaxed and the pus from the uterus escapes in the anterior part of the vagina. This is expelled when the cow lies down or while urinating or defaecating. On vaginal exploration about  $\frac{1}{4}$  litre pus will be found in the cranial portion of the vagina. The pus is usually thick, mucoid, creamy and yellow, white or greenish-grey in colour. On rectal palpation the uterine wall is usually felt thickened, flaccid and atonic. The pus that distends the uterus may simulate early pregnancy [Fig. 76]. But the consistency of the pus is heavy and watery like that of placental fluids. Slipping of the foetal membranes which is characteristic of pregnancy is not felt. The foetus, cotyledons, and enlarged uterine artery as in normal pregnancy are not palpable in pyometra.

## Diagnosis

The histological picture in pyometra cases is typical of suppurative process and includes fibroblastic proliferation and thickening of stroma, infiltration of glands by polymorphs and few macrophages. The glands contain pus, debris and organisms. Exfoliation of lining mucosa and fibrotic replacement of glands is evident. Such lesions were observed by Baur (1951), Simon *et al* (1967) in cows and by Velhankar *et al* (1965) in buffaloes.

## Prognosis

Beneficial results are only possible in case treatment is done earlier. In long standing or chronic cases, the endometrium is destroyed, the uterine wall undergoes fibrotic changes. Cases which have existed for 60 to 120 days only recovery and conception are more likely than in chronic cases. The type of organism and its effect on the endometrium and uterus have an important role. In incurable cases *C. pyogenes* is commonly found. Cases with excessive quantities of pus resulting in distended condition of the uterus, and marked lack of tone, seldom results in recovery. When the condition is associated with severe peri or para-metritis, prognosis is utterly hopeless.

## Treatment

The treatment of pyometra cases in cows and buffaloes mainly consists of an initial stimulus to the uterus and specially to cervix to cause dilatation, so that it is possible for the uterus to evacuate its contents through the relaxed cervix. The dilatation of cervix occurs at oestrus or in induced oestrus. Manual removal of the corpus luteum is not considered as proper, since such an

enucleation in the presence of an infective condition of the uterus may result in the flare up of the infection. The difficulties are always experienced in the enucleation of the well embedded or retained corpus luteum in cases of pyometra in the cow and buffalo which always results in trauma to the ovary. In such cases, if there is presence of pyogenic organisms in the oviducts it may develop in severe adhesions. Administration of oestrogens is therefore considered advisable since stimulation to the uterus and cervix is necessary for prolonged period for evacuation of the pus and for regeneration of the endometrium. Stilboestrol in the dose of 25 to 50 mgm or oestradiol 5 to 10 mgm may be given intra-masculary. Higher and repeated doses at weekly intervals may be necessary depending on the degree of the condition. Stilboestrol may act within 2 to 3 days and cause evacuation of pus through the relaxed cervix. Rectal palpation may show contracted condition of the uterus in about 10 to 15 days. As far as possible repeated treatment with oestrogenic compounds should be avoided so as to eliminate the danger of ovaries becoming cystic.

Antibiotics have to be used concurrently with oestrogens to overcome infection. It is preferable to use antibiotics both parenterally and intra uterine when the cervix is relaxed. In certain cases recovery is only possible after the resumption of regular oestrous cycles.

Massage of the uterus per rectum is not advisable as due to pressure, the contents of the uterus may be pressed further into lumen of oviducts. Excessive massage may also lead to perimetritis and adhesions.

Administration of pituitrin is not found beneficial on account of its transient effect.

In cases where there is no response, uterine wall may be found thickened, fibrosed or atrophied. In some cases, the internal os of the cervix may be found to have become hard and indurated which prevents evacuation of pus.

After the apparent recovery the cow should be watched at two to three normal oestrous periods. Breeding should only be done in case oestrus discharge is normal.

Pyometra is likely to produce severe damage to the endometrium which may prevent conception even if pus is completely evacuated. Considering the economics and uncertainty of cure, farmers usually prefer to sell such animals for slaughter. It is therefore necessary to give a fair idea to the farmers before undertaking such cases for treatment. The best way to avoid occurrence of pyometra is to give prompt attention to cases in which there is voidance of mucopurulent discharge, from the uterus. Syphoning out the putrid content on relaxation of the cervix and flushing the uterus with mild warm antiseptic solution is considered beneficial. Use of diluted hydrogen peroxide intra-uterine has given good results in cleaning the debris accumulated in the uterus concurrently followed by broad spectrum antibiotics (Sane *et al*, 1964).

### Mucometra or Hydrometra

The condition is occasionally met with in cows. It is characterised by cystic degeneration of the endometrium and atrophy of the uterine wall with accumulation of thin to viscid mucus in the uterus in varying quantities from 30 ml. to 5 litres. (Fig. 77).

Mucometra is also observed in heifers or cows having arrested development of the Mullerian duct system, in which,

part of the uterus, cervix or vagina may be defective or missing. Persistent hymen or uterus unicornis may result in locking up of the uterine contents, resulting in distention of one or both the horns with watery, viscous or even coagulated masses of mucus and cellular debris which may be confused with pregnancy. In such heifers and cows, the ovaries are usually normal and oestrus is exhibited in the usual manner. In mucometra there is no infection unless it is introduced during service or by insertion of pipettes during treatment to the uterus.

Diagnosis of Mucometra can be differentiated from pregnancy by a very thorough examination of the genital tract. There is absence of slipping of the foetal membranes. There may be presence of anomalies in the uterus, vagina and cervix. Ovaries may contain a single or multiple small cysts. Middle uterine artery is neither enlarged nor fremitus can be felt. No foetus or cotyledons can be felt. Very firm or gum like mucus in one horn may complicate diagnosis. The uterine wall is usually very thin and lacks tone as compared to the normal pregnant uterus. Progressive changes of normal pregnancy are absent.

Cows with mucometra are usually incurable. From hereditary standpoint, it is not at all advisable to breed heifers or cows having mucometra or hydrometra.

### Perimetritis and parametritis

These conditions are occasionally met with in cows and buffaloes and are characterised by adhesions between the uterus and broad ligament to the other pelvic and abdominal organs. [Figs. 78, 79]. These adhesions are usually secondary to one of the following conditions:

(1) Very severe septic metritis.

(2) Perforation of the uterus due to penetration by a catheter or due to heavy douching of irritable antiseptics.

(3) Rupture of the rectum due to rough handling during rectal examination.

(4) Occasional severe haemorrhage, trauma, or rupture of uterus during parturition due to torso uteri, embryotomy, forced extraction, severe cervical or vaginal lacerations, caesarian section, an extra uterine foetus, excessive bleeding following enucleation of a corpus luteum, excessive massage, manipulation of an infected uterus, forcible extraction of retained placenta and occasionally it may even be caused due to diffuse peritonitis secondary to tapping of the rumen or traumatic reticulitis. Tuberculosis of genital organs may lead to perimetritis and parametritis.

The lesions may be found from a few thin fibrous strands of connective tissue to firm adhesions developing between the uterus, broad ligament, rumen, omentum, intestines, rectum, urinary bladder or other organs. The adhesions may be either diffuse or localised. Abscesses may be found occasionally in the adhesions around the ovaries or broad ligaments or even between the uterus and the rectum. The abscesses may be small or large. The perimetrial abscesses may in severe cases rupture into the rectum or bladder or may extend through the pelvic cavity to rupture alongside of vulval lips.

During the acute stage of perimetritis or parametritis, the usual symptoms of perimetritis such as anorexia, arched-back, slow stiff gait, frequent painful micturition, and defecation, decreased

rumen activity and slack ruminations are observed. The temperature may rise for a day or so or in some cases there may be no elevation. The pulse rate and respirations are increased. An "expiratory grunt" may be heard, abdomen may become tense with ruminant tympany. Diarrhoea may set in. Hair coat may progressively become rough. In lactating cows there is perceptible drop in the milk yield. In acute cases if rectal examination is done, the animal may show evidence of pain, tenesmus, grunting, and an anxious expression. In this acute stage fibrinous adhesions can be broken with ease. In case such symptoms are exhibited, rectal examination should be immediately suspended to avoid further spread of inflammatory processes. If the cow overcomes the acute stage, adhesions may become chronic. Affected areas are walled and result in encapsulated abscesses, which can readily be felt on rectal palpation. Perimetritis and perimetrial abscesses may sometimes be confused with tumours or fat necrosis.

### Prognosis

Prognosis varies according to the severity of the condition. Slight adhesions may not cause any trouble and these can be broken by hand per rectum. In chronic cases with severe extensive adhesions, the prognosis is usually very poor, especially when adhesions form in-between ovaries and oviducts.

### Treatment

Treatment of the affected cases is more or less on the same lines as in pyometra. Antibiotics, sulphanilamides, oestrogenic compounds, nitrofurantoin compounds and cortisones are administered according to the severity of the condition.

### **Uterine abscesses**

Localised abscesses in the uterine cavity are of rare occurrence. The shape is usually oval or round and on palpation a tense firm swelling is felt. The size may vary from 1 cm to 15 cm in diameter. The abscess will change the normal outline of the uterine cornua. Such abscesses may usually occur as a sequelae to severe metritis, after forcible extraction of retained placenta, injury to the uterine wall due to improper penetration of the inseminating catheter or by rough use of instruments during embryotomy operations. Trauma caused to the deeper structure especially during infective condition of the uterus permitting infection to penetrate through the endometrium may lead to uterine abscess. Abscess in the uterine wall can be easily diagnosed on rectal examination. It should be differentiated from a cyst, haematoma or tumour. Perimetritis and adhesions may develop round about the abscess. Symptoms are not very definite. The cow may not conceive or she may abort even if she settles to service. In rare instances adhesions may form between the abscess and the rectum, bladder or vagina and it may rupture in these parts.

Treatment with antibiotics parenterally during early stages may prove beneficial.

### **Sclerotic metritis**

This may occur as a sequelae to severe chronic metritis which has resulted in complete destruction of the endometrium. The endometrium is usually transformed into a thick dense layer of connective tissue with foci of infection and secretion of purulent exudate in the uterus. The condition often involves the cervix. The thickened

indurated condition of the uterus usually follows severe cases of chronic pyometra where infection exists for prolonged periods or it may follow foetal maceration or severe septic metritis. The endometrium and uterine caruncles are destroyed and as such the affected animals become sterile. On rectal palpation the uterus will be felt as very hard firm structure, more or less like a cartilage or dense fibrous tissue. There is enlargement of the uterus and the chronic exudate may appear at the cervix. The cervix becomes thickened and indurated. In case there is foetal maceration bones may still be felt in the uterus. The cow fails to show oestrus and the corpus luteum is found deeply embedded in the ovary.

### **Prognosis**

Prognosis is never hopeful and as such culling is advised.

### **Uterine tumours**

These are not very common in cows and buffaloes. Lagerlof and Boyd (1953) reported on their studies on 6286 bovine genital organs that uterine tumours were detected only in 44 cases. These included 12 fibromyomas, 7 fibromas, 15 leiomyomas, 1 scirrhous adenocarcinoma, 1 lympho sarcoma, 1 spindle cell sarcoma and 7 undiagnosed tumours. A solitary fibroma may not interfere with fertility. Williams (1913) described 1 case of fibroma in a cow, that produced several calves in the unaffected horn. Wadsworth (1952) reported fibromas, fibromyomas, lipomas leiomyo-sarcoma, carcinomas and adeno carcinomas of the uterus in the cow. Most of the uterine tumours in bovines are benign. Metastatic tumours may develop occasionally in the uterus. Lymphosarcoma in cattle is commonly met with in the Midwest,

and in such cases involvement of the uterus, is occasionally observed. Sane *et al* (1958) recorded only one case of fibroid close to cervix in a buffalo. There were some difficulties in settling that buffalo to service but on removal of the fibroid, there was normal conception, gestation and parturition.

Infertility or sterility caused due to uterine tumours is dependent on the type of tumour, its location, size, shape, and extent to which it is interfering with the normal functioning of the uterus. Effect is therefore very variable, mostly depending on its nature, benign or malignant.

### Affections of the cervix

#### CERVICITIS

In the cow, inflammation of the cervix is commonly observed and is usually associated or coexistent with metritis. It may frequently follow abnormal parturitions such as abortions, premature birth, dystockia where forceful extraction of the foetus or manipulation of instruments during embryotomy may cause cervical tear and lacerations. Retained placenta and post partum metritis may act as contributory factors. Infec-

In older cows prolapse of the external transverse cervical rings, is a possible factor causing cervicitis. The prolapsed rings become chronically thickened, fibrosed and blood circulation to the epithelium may become poor giving ample scope for the infection to establish. Purulent vaginitis or vaginitis associated with pneumovagina may cause cervicitis.

#### Incidence

In cows, Quinlan (1929) described 64 cases of inflammation of genital tract out of which 34 were of cervicitis. He recorded 18 cases of chronic cervicitis, 3 catarrhal cervicitis and one chronic purulent cervicitis.

In buffaloes, based on clinical examinations, Bhattacharya *et al* (1954) recorded the incidence of cervicitis as 10.52%, Sane *et al* (1958) 1%, Malik *et al* (1960) 9.9%, and Bhandari (1963) reported incidence of cervicitis, on histopathological studies as 26.61% in Murra buffaloes.



matically act as curative to the cervix. Treatment with antibiotics, oestrogen therapy, cortisones, and lugol's solution of iodine are found to give beneficial results.

Occurrence of cervicitis may be checked by preventing possibilities of ascending infection, trauma during dystocia, forced extraction of the foetus, and rough use of catheters during the course of insemination and intra-uterine treatment.

### Cysts of the cervix

These are occasionally observed in the cervix of cows and buffaloes. They are called as Nabothian cysts or Nabothian follicles, as in the human. The cysts vary in size from 1.25 to 7.5 cm in diameter. Apparently these are retention cysts of the cervical glands. When these are large enough, they become palpable on rectal examination as movable or fluctuating mass in the cervix. Portion of the cyst may appear at the os uterus and are visible on vaginal examination. Nabothian cysts rarely cause infertility. However when they are large enough they may hold mucus or debris in the uterus or prevent proper closure of the cervix during pregnancy (Sane *et al*, 1969).

The cyst can be differentiated from an abscess. The cyst is usually thin walled and covered with mucous membrane, whereas the abscess in the deeper tissues of the cervix has a thicker and more fibrous wall. Occasionally cystic dilation of the cervix in the cow may be due to arrested development or fusion of the Mullerian ducts. This is associated with "White Heifer disease" and prognosis in such cases is invariably poor.

### Stenosis or Obstruction of the cervix

This is rare in the cow. In some heifers, the cervical canal is small and tortuous and it is difficult to pass insemination pipette. When such heifers fail to conceive, stenosis of the cervix may be suspected. Olds and Seath (1954) reported on their studies of 1711 heifers and 11,112 cows that the cervix was impenetrable to an inseminating pipette in 11.7% and 1.1% respectively. Their studies indicated that this condition should not be considered as abnormal causing infertility.

### Tumours of the bovine cervix

These are rare. Wadsworth (1952) has described fibromas, fibrosarcomas, leiomyomas and carcinomas affecting the cervix. Most of these tumours were of benign type. Very few may be malignant and may metastasise to adjoining organs. Operative treatment is very difficult and doubtful.

### Vaginitis

#### AETIOLOGY

The condition may be primary or secondary. The secondary condition is usually associated with metritis and cervicitis. Vaginitis may be caused due to trauma, lacerations and infections which may occur at the time of abortion, dystocia, embryotomy, manual removal of the placenta, prolapse of the vagina and as a consequence of post-partum metritis. In older cows, sinking of the anus and stretching of the vulva into horizontal position may cause atrophy of the vulval lips, often resulting in pneumovagina, causing severe contamination of the vaginal passage by faecal matter, urine, air, debris and infections of various types which cannot

be readily overcome since the uterus and cervix have pulled the vagina forward and downward into the abdominal cavity. Occasionally vagina may be inflamed due to forcible coitus but this is usually of mild type and does not require any treatment. Small abscesses may develop in the vaginal mucosa (Fig. 80) or in the Gartner's ducts. Vaginitis may occur as a result of using irritant antiseptic solutions for douching the uterus or vagina. It may also occur due to dirty hands and infective instruments used during treatment. Majority of the cases of vaginitis are due to non-specific infections such as *Streptococci*, *C. pyogenes*, and Coliform organisms. Vaginitis may occur due to specific infections such as vesicular venereal disease, *Vibriosis* and *Trichomoniasis*.

### Symptoms

Muco-purulent yellow greyish pus is usually discharged from the vulva at irregular intervals which mats the vulvar hairs and soils the tail and buttocks. When vagina is dilated by means of speculum, a fair amount of exudate is often observed on the floor of the vagina. There is congestion, oedema and inflammation. The degree of inflammation is dependent on the severity of infection. In very severe affections, the cow may show irritation, tenesmus and pain. Uncomplicated forms of mild vaginitis may not prevent conception. A severe infection of the vaginal passage and of the external os of the cervix in which there is accumulation of pus and debris and also having an altered pH may cause rapid destruction of spermatozoa that may interfere with fertility. In old cows having pneumovagina, there is usually an accumulation of material often associated with atony of the uterus and the genitalia hangs down in

the abdominal cavity due to heavy accumulation of the mixed materials. The cervix may permit some of the infectious material to pass on to the uterus where by it may cause endometritis resulting in infertility or sterility. The cows may repeat to service a number of times. On proper treatment of vulva it may regain its normal power to close the vaginal opening whereby vaginitis can clear up in a short time. The external os of the cervix is usually involved in almost all cases of vaginitis. In metritis and cervicitis infective discharges always accumulate in the vagina and the vaginal inflammation becomes secondary to this condition. Secondary stenosis and even atresia of the vagina may follow in rare instances.

### Prognosis

In majority of the cases of vaginitis recovery occurs in a short period even without treatment. In infective conditions however the recovery is dependent on the severity of infection and treatment adopted in good time. Prognosis is usually very poor in the presence of stenosis or atresia of the vagina.

### Treatment

It consists of draining of the discharge that has accumulated in the vaginal cavity followed by antibiotic therapy. Douching with antiseptic solutions has to be done very cautiously to prevent escape of pus from vagina to cervix and further to uterus. If at all douching has to be done acriflavine solution 1:3000 may be used. Dettol solution, sodium bicarbonate solution and saline solutions may give beneficial results. Care has to be taken to find if vaginitis is co-existent with cervicitis and metritis in which case concurrently it is also necessary to extend treatment to the

uterus. Antibiotics such as penicillin, streptomycin, tetracyclins and oxytetracylin in aqueous solution or creams give favourable results. Favourable results by successive treatment from 3 to 7 days have been claimed by use of antibiotics, depending on the type and severity of infection. In cases of pneumovagina, Caslick's operation is necessary followed by use of antibiotics intrauterine. In cases of vaginitis, the animal has a tendency to rub its tail on the vulva leading to severe irritation. It is better to secure the tail to one side.

### Cysts of the vagina

They are not very common. These may occur in two sites. Cysts of Gartner's duct on the floor of the vagina are usually of the size of 1.25 cm in width. Cysts may be found along the course of the ducts. They are harmless and seldom require any treatment. Cystic condition of the Bartholin's glands is observed more often. These are formed due to atresia of the duct of the gland that usually opens on the lateral wall of the vagina or vulva at the vulvo vaginal border. This small paired Bartholin's gland secretes a clear mucus. Obstruction of the duct is usually due to a trauma or severe inflammatory condition such as necrotic vaginitis which may occur during parturition. When the cystic gland assumes a bigger size as that of 5 cm in diameter, it usually becomes detectable between the vulval lips when the cow is in lying down position, as a round pink well elevated area which is often mistaken for the beginning of a prolapse of the vagina. It is very rarely seen in heifers. It is almost invariably unilateral and is seen in older cows. It does not interfere with conception. However, when it becomes large it may protrude beyond the

vulval lips, get soiled, and possibly catch infection which may spread to vaginal passage. The cysts may be opened and contents evacuated.

### Tumours of the vagina

These include fibromas, fibrosarcomas, and carcinomas. The occurrence of vaginal tumours in cows is rare. Most of the tumours are benign pedunculated and may cause obstruction during parturition. Surgical removal becomes essential when they protrude through the vulva.

### Vulvitis

Vulvitis is usually characterised by granular venereal disease lesions of which may cause inflammation, swelling, pain, straining and the cow may refuse copulation. In severe cases, vulval lips are swollen and there is presence of mucopurulent discharge as is found in vesicular venereal disease. Injuries and lacerations of the vulva may cause considerable pain. The swelling may reduce the size of the vulval opening. Wounds should be properly sutured and treated to prevent occurrence of pneumo-vagina. Care is necessary to keep flies off. In general, treatment consists of antibiotic dressing and dusting with boriodoform.

### Tumours of the vulva

Fibromas, fibrosarcomas, angiomas, carcinomas and fibro-papillomas have been reported by Williams (1913), Wadsworth (1952) and McEntee (1950). McEntee (1950) reported 28 cases of fibro papillomas on the vulva and vagina of cows. A solitary case of carcinoma of vulval lips was recorded in a Gir Cow (Sane *et al.*, 1976). McEntee concluded from his studies that as this

was discovered in several animals from the same premises the fibropapilloma is a transmissible tumour of the external genital organs in cattle. He describes that it is similar to common wart, and undergoes spontaneous regression. He considers that this is similar to papilloma found on the body of young cattle caused by a virus.

### Repeat breeding

In most countries of the world, stock breeders have to face problem of repeat breeding in cows, to a lesser or greater degree. In a fairly well maintained herd, a cow on an average requires one to two services per conception.

A repeat breeding cow is one which has normal reproductive tract with normal oestrous cycles but which does not settle even to repeated services by a fertile bull or inseminated by a good sample of semen. These can neither be considered as sterile nor anyone can say as to when they will settle to service.

The factors responsible for repeat breeding have been reviewed by Casida (1961), Jainudeen (1965), Olds (1969), Roberts (1971) and Pendse (1974).

Critical experiments carried out by Tanabe and Casida (1949), Christian *et al* (1951), Tanabe and Almquist (1953), Kidder *et al* (1954) and Bearden (1954) showed comparison between repeat breeding cows, repeat breeding heifers and normal virgin heifers bred to bulls of high or low fertility. In these experiments the repeat breeding cows and heifers were bred to fertile bulls at least four times or even more.

Tanabe and Casida (1949) and Christian *et al* (1951) reported on the fate of embryos in repeat breeding cows as follows:

Sr. No.	Item	Number of cows studied	Percent of Potential Embryos
1.	Fertilization failure, ovulation failure and tubal obstructions (about 50% due to each cause).	247	6.0
	No apparent cause.	129	39.3%
2.	Embryonic death.	—	32.5*
3.	Normal embryos at 34 days.	168	22.2
			100.0%

\*It was estimated that 54.4% of the fertilized ova and embryos died. The fertilization rate was 54.7%.

Tanabe and Almquist (1953) reported as follows on the fate of potential embryos in repeat breeding heifers.

	Number of cows studied	Percent of Potential Embryos
Fertilization failure, ovulation failure, congenital or acquired tubal obstructions.	96	13.5
Degenerated or ruptured ova.	71	9.5
No apparent cause	71	23.8
Embryonic death	—	22.6*
Normal Embryos at 34 days.	36	30.6
		100.0

\*It was estimated that 54.1% of the fertilized ova and embryos died. The fertilization rate was 54.7%.

The preceding two summaries outline the problem in 'repeat breeding' cattle. The following reports by Kidder *et al* (1954) and Bearden (1954) were designed to test similar factors by breeding normal virgin heifers to bulls of high fertility or low fertility by their non-return records in actual service in artificial insemination. They serve also as a standard by which the above figures in 'repeat breeding' cows and heifers may be evaluated.

Kidder *et al* (1954) bred virgin heifers to high, medium and low fertile bulls, with the following results:—

	Number of heifers studied	Percent of potential embryos
Fertilization failure, Abnormalities or pathology of genital tract.	74	2.7
Defective ova (usually empty zonapellucida)	61	9.5
No apparent cause	61	12.2
Embryonic death	—	16.0*
Normal embryos at 60 to 90 days estimated from non return results.		59.6
		100.0

\*It was estimated that 21% of the fertilised ova and embryos died. The fertilization rate was 85%.

Virgin Heifers Bred To :	Fertilization rate percent	Estimated embryonic deaths percent
High fertile bulls	100	25.5
Medium fertile bulls	82.1	—
Low fertile bulls	71.4	14.9

Bearden (1954) bred virgin heifers to high and low fertile bulls.

	Number of heifers studied	Percent of potential embryos
Fertilization failure, abnormalities or pathology of genital tract	110	3.6
Defective ova (usually empty zona pellucida)	110	1.8
Non apparent cause	85	13.2
Embryonic death	—	15.3*
Normal embryos at 33 days	55	66.1
		100.0

\*It was estimated that 20.7% of the fertilized ova and embryos died. The fertilization rate was 86.8%.

In his experiment high fertility bulls (74 to 76%), (non returns at 60 — 90 days) and low fertility bulls (41 to 63%) were bred to the virgin heifers with the following results.

Virgin Heifers Bred To :	avoided thoughtless	avoided thoughtless	avoided thoughtless
	avoided thoughtless	avoided thoughtless	avoided thoughtless

low fertile bulls was not significant because the numbers of heifers used in the experiment were not sufficiently large. No endometritis was found in these non-pregnant heifers at the time of slaughter.

It appears from the reports of Bearden (1954) and Kidder *et al* (1954) on virgin heifers that there is no significant difference between high and low fertile bulls in respect of the number of embryonic deaths in each group which shows that the bull plays little part in the problem of repeat breeders. However they observed significant difference between two groups of bulls in fertilization rates. It is possible to determine high or low fertility bulls only by use of their semen through artificial insemination. Tanabe and Casida (1949) recorded a fertility rate of 51.7% in repeat breeding cows which were inseminated with diluted semen without antibiotics, whereas when semen was used with antibiotics a fertility rate of 66.7% was recorded by Tanabe and Almqvist (1953) in repeat breeding heifers. Fresh semen was used for insemination to maintain high concentration of viable sperms. The causative factors for repeat breeding cows fall into two main categories: (1) Failure of fertilization, (2) Early embryonic death.

In a field investigation of 152 repeat breeding crossbreds, Deshpande and Deopurkar (1981) reported following factors and incidence — (i) No proper detection and non-synchronization 27.4%, (ii) Repeating at regular intervals 7.6%, (iii) Repeating at irregular intervals 6.9%, (iv) Metritis 10.7%, (v) Genital abnormalities and adhesions 10.7%, (vi) Cystic ovaries 6.1%.

## (1) FAILURE OF FERTILIZATION

This may be due to abnormalities in ovulation or morphological defects in the ovum or spermatozoa. Obstruction of the oviducts may cause failure of fertilization. About 35 to 40 per cent of repeat breeding cows are repeaters due to the above causative factors as compared to 5 to 25% repeat breeders in virgin heifers (Roberts 1956).

### Abnormalities in ovulation

These may be due to failure of ovulation, delayed ovulation or ovulation of two or more ova at one oestrus. Casida (1953) reported on his observations that failure of ovulation occurred in about 3 to 5% of repeat breeders. It is difficult to diagnose delayed ovulation unless repeated examinations are done to palpate the ovary.

It can be concluded from the data collected by Casida (1953) that from 15 to 11% of the ova are likely to be lost as a result of the ova being caught in the collapsing walls of ovisacs or due to being slipped in the peritoneal cavity, and failure to enter the fimbriated extremity of the fallopian tube.

Kidder *et al* (1952) reported an incidence of twin ovulations in a herd of cows to be 13.1% whereas the actual incidence of twinning in the same lot was only 1.9%.

Henning (1939) reported in sheep that live foetuses represented 92% of the corpora lutea in cases of single ovulations, 71% in double ovulations and 57% in triple ovulations which evidently shows that failure of fertilization or early embryonic deaths are of common occurrence in multiple ovulations. High rate of embryonic mortality is commonly observed in cases of superovulated ova. In multiple ovula-

tions, high rate of loss may possibly be due to failure of fertilization.

### Morphological defects of the ova

Defective or pathological ovum, ageing of the ovum, or other factors may prevent fertilization of the ovum. Kidder *et al* (1952), Tanabe and Almquist (1953), Bearden (1954) reported that 2 to 9.5% of the recovered ova showed gross abnormalities. The most common defect observed was empty zona pellucida. Degenerative changes and rupture or shrinkage of ova were the other defects. The life of an unfertilized ovum is just for few hours only and it is therefore necessary that fertilization should occur before the viability is decreased. Casida (1950) reported that 75% of the ova were fertilized when inseminations were done within 2 to 8 hours after ovulation, 60% when inseminations were done in 9 to 12 hours, 25% in 14 to 16 hours, 40% in 18 to 20 hours and no fertilizations occurred when inseminations were

insemination, as cows to be served naturally will refuse to stand to the bull when oestrus stage has passed. Unobservable changes are also detected due to inherent or physiological defects occurring during its development.

### Inability of the sperm to fertilize a viable ovum

This seems to be the most common cause for failure of fertilization. In virgin heifers about 12% of the repeat breeders are due to the inability of the sperm to fertilize, whereas it may be between 24 to 40% in repeat breeding cows. In heifers bred to highly fertile bulls, failure of fertilization was 0 to 5% whereas with less fertile bulls the failure was to the extent of 24 to 30% even though, the semen quality of these bulls was practically similar to that of high fertile bulls (Roberts, 1956). Inflammatory conditions of the genital tract may cause adverse effect on the spermatozoa and fertilization. Inflammatory conditions of the genital tract

inflammatory conditions. Hormonal and nutritional may be other causes. In repeat breeders, 20 to 30% loss is due to embryonic death, and 50% loss of fertilized ovum. In heifers the percentage is half of this.

### **Congenital or genetic defects of the fertilized ova or embryos**

This can be better studied on multiparous animals since number of ova shed are subjected to the same environmental conditions of the genital tract. Failure of fertilization or death of the embryo is probably due to inborn defects or due to difference between each ovum or embryo in respect of their endurance, viability, and capacity for growth. Such perfect ova survive whereas the poor ones perish. Casida (1953) reported fertilization rate of 91.3% in rabbits, and 82% in pigs. Of these embryos which survive to term were 50.9% in rabbit and 55.8% in pigs. Thus the estimated loss of embryos or fertilized ova was 41.2% in rabbits and 36.7% in pigs. In swine early embryonic death in certain zygotes is not necessarily be associated with infection in the genital tract. It also appears that it does not seem to be associated with high litter numbers or with different levels of nutrition. It is reasonable to believe that the cause lies in some inherent elements in the fertilized ova. In an experiment normal virgin heifers were bred to highly fertile bulls and even though 96% of the ova were fertilized, 10% succumbed before 33 days and another 10% between 60 to 90 days (Bearden, 1954). Casida (1950) stated that there was only 8 to 11% difference between the two groups, indicating that repeat breeding may be an inherited factor. Environment during developmental stage of fertilized

ova has great importance. Christion *et al* (1951) showed that cross breeding or using bulls of another breed on repeat breeding cows did not increase conception rates. Study of lethal genes as a probable cause has to be considered.

Production of abnormal ova may be related to anomalies in the development of Graafian follicles. This is substantiated by the high death rate of fertilized ova from multiple ovulations or from artificial super ovulations in uniparous animals. Multiple ovulations are somewhat inherited and as such this genetic condition may be related to the hormonal control of ovulation and to the production of normal, abnormal or non viable ova. Salisbury *et al* (1952) reported marked differences between bulls in the percentage of embryonic deaths. Foote and Bratton (1952) demonstrated that 112,312 cows bred at first service by A.I. with diluted semen without antibiotics had non return rate of 79.1%, 64.1% and 60.3% at 28 to 35 days, 60 to 90 days and 150 to 180 days respectively. In another lot of 233, 354 first inseminations done with additions of antibiotics to the diluted semen, the respective non return rates obtained were 82.5%, 73% and 69.7%. This evidently has shown that the addition of antibiotics decreased early embryonic deaths. Similar observations have been made by Erb and Flerchinger (1951). Long return intervals were markedly associated with low fertility of those cows at second service, indicating vibriosis as the probable cause.

### **Diseased condition of the embryos**

This is probably the most common specific cause of early embryonic death. Enzootic causes known so far include Trichomoniasis, Vibriosis and Brucello-



sis. Tanabe and Casida (1949) reported in respect of Brucellosis, an embryonic mortality of 77.9% in the positive group and 60.1%, in the negative group of repeat breeders. An increase in severity of endometritis in Brucella positive cows was noted by Brus (1954) and Kampelmacher (1954). Addition of the antibiotics to the semen has raised the non-return percentage by about 10%. Miscellaneous infections likely to cause chronic endometritis, usually follow delayed involution of the uterus due to pathological conditions of the uterine tract during parturition and post partum period. Probably it appears that these infections act as causative factors in the early death of ovum or embryo. The role of viral infections and their effects on early bovine embryos is of considerable importance. Published reports indicate that the administration of modified Hog-cholera virus to susceptible sows 10 to 16 days after breeding resulted in early embryonic death and in deformed piglets. Similar effects have been recorded due to measles virus on the human embryo.

Trautman (1954) reported that cows severely affected with granular venereal disease showed non-return rate 10% lower than that of cattle which were not affected.

#### **Abnormal environment of the oviducts and uterus**

Disturbed environment of the oviducts and uterus may play a great role in the early death of the ovum. Tanabe and Almquist (1953) reported that 55.5% of 36 repeat breeding heifers came into oestrus prior to 30 days after their last service. Tanabe and Casida (1949) reported that about 50% or 27 out of 53 repeat breeding cows came

back to oestrus prior to 32 days. Bearden (1954) reported that eight out of 26 heifers bred to low fertile bulls returned to oestrus by 28 days. Seven of these were between 17 and 20 days. It appears from the above report that a certain percentage of fertilised ova die before the 10th day of the cycle and the cow repeats to oestrus at the regular cycle interval, while in others the zygote dies after the middle of the cycle and the return to oestrus may be delayed. Hawk *et al* (1955) reported that in repeat breeding cows, major number of deaths of early embryos occurred between 16 and 34 days. However it was not possible for them to determine as to the causative factors. Some believe that progesterone deficiency creates unfavourable environment in the uterus for nidation of the zygote. This belief is based on the fact that the injection of progesterone and LH causes early ovulation and also rapid transport of the ovum through the oviduct and it is likely that an excess of these hormones might prove harmful. Histologic evidence is against a lack of progesterone as a cause of embryonic death (Laing, 1949). Casida (1953) showed that progesterone administered during early stage of pregnancy in rabbit had no effect on reducing embryonic deaths. Oestrogen administration during oestrus has been shown to delay ovulation and have either greatly slowed the rate of passage of the ovum through oviducts or locked the ova in the oviducts. Laing (1952) indicated the possibility that isoimmunisation may possibly be a factor in early embryonic deaths. The relationship of the plane of nutrition to early embryonic deaths in cows is not clearly known. However, Casida (1953) cited that in swine, a lack of Vitamin B12, animal protein and full feed for gilts during their breeding period re-

sulted in a higher embryonic deaths than sows and gilts that were fed on Vitamin B12 supplement and kept on a low plane of nutrition during the breeding season and early part of gestation.

### **Management problems and infertility in cows**

For maintenance of good fertility in a herd, careful attention has to be paid to the maintenance of accurate records in respect of the day to day observations, the type of oestrus in each cow, and service done at optimum time by natural or by artificial insemination method. Management practices such as adequate feeding and watering, exercise, stable hygiene, protection from venereal infections and care during parturition and puerperium period go a long way in maintenance of good fertility. Improper and negligent managerial regime is one of the major causes of infertility in cattle.

### **Recommendations for obtaining a good breeding record in a herd**

#### **I. GENETIC PRACTICES**

As far as possible avoid inbreeding to prevent likely concentration of undesirable hereditary traits of recessive type. When defects become evident the animal should be culled.

#### **II. MANAGERIAL PRACTICE**

An up to date breeding record for every animal should be maintained. The records should include rate of growth upto puberty age, date of first oestrus, subsequent dates of oestrus, date of service, the name of the bull, date of early pregnancy diagnosis, date of calving, any abnormalities encountered during parturition, time taken for expulsion of after-birth, type of lochial discharge, and time when such dis-

charge has ceased, size and weight of the calves and any treatment done for sub fertility and pathological conditions of genital tract.

It is advisable that a farmer should watch his cows, on their respective standings in the stable early in the morning before they are let out for grazing. This will enable him to observe the type of discharge voided by each and every cow and to detect if the discharge is healthy. It is better that the herd should be let out twice daily and close observations made on all cows for about half an hour during morning and evening hours. Such observations are necessary so that heats may not be overlooked.

Even cows that have calved normally should not be bred within 60 days after parturition since complete involution of the uterus may not have occurred by this period. With history of abortion or retained after birth, cow should not be bred within 3 to 4 months. A group of cows should be assigned to a particular bull. If the cow repeats to services on more than two to three occasions, she should be bred by artificial insemination only, on confirmation of clinical evidence that she is clean. The practice of using the bull alternately may spread venereal or other miscellaneous types of infections. In a composite herd, if a cow repeats to services to a particular bull, other bulls may be used alternatively in case the cow is found clean. Such a practice will lead to enhance conception rate.

Service should be offered at the most appropriate time during the heat period, i.e. at the midoestrus to end of oestrus. Service done 6 to 12 hours after the end of oestrus may result in lowered conception rate. Cows which are detected on heat during morning

hours should be bred the same day during afternoon hours and cows which show heat during afternoon or evening hours should be bred next morning.

Sires and dams may be of different levels of fertility. Howsoever considering that the sire is to be mated for large number of conceptions, sire of high fertility should necessarily be used.

Well developed heifers may be bred at an early age after they have attained weight from 250 kg. onwards which they usually acquire between 2 to 3 years age in Indian breeds on good plane of nutrition.

In herds where coital infections are prevalent, it is desirable to breed by artificial insemination.

All pregnant cows should be dried off two months before calving. They should be observed closely during parturition. As far as possible avoid surgical operations, transport either by conveyance or on foot and any such conditions producing stress and fatigue during latter third of pregnancy period.

### III. NUTRITIONAL PRACTICES

Newly born calves should be handled very carefully. Attention should be paid to find if they have received proper feeding of colostrum. Care should be taken to feed them adequately during growth period.

been recorded by Sane *et al* (1964) on various breeds of draught and milch type of cows.

Pasture grazing decidedly has beneficial effects on the health of the herd.

### IV. MEASURES FOR SEXUAL HEALTH CONTROL

Care should be taken to eliminate venereal infections like Brucellosis, Vibriosis, Trichomoniasis.

Cows showing abnormal type of genital discharge, oestrous periods of frequent or continuous type, absence of oestrus for about 3 months after calving or requiring two to three services from a fertile bull for conception, should all be examined by a veterinarian. Uterine infections, and other diseases or lesions of the genital tract if diagnosed early are amicable to treatment.

Cows that are served should be subjected to pregnancy diagnosis at 6 weeks, and thereafter every 60 days, to ascertain that there is progressive foetal development. Such examinations have many advantages to detect if any cow that was served has not settled to services and also which has not repeated to oestrus at regular periods. Treatment to such cows is possible to avoid expenditure on maintenance. Cows which may not have settled to service but which may have

per rectum in 2 to 3 weeks time to find if complete involution of the uterus has occurred. Repeat examination after 60 days should be done to find if the animal is fit for breeding.

Animal showing inherited defects of the reproductive tract or gross pathological conditions of the genital tract should be culled immediately.

In the normal course 13 to 17 services per conception or 60% conceptions should occur on 1st service and by the third service about 90% of the cows should remain pregnant on a well managed high fertile herd. About 5 to 10% yearly loss of cows is due to reproductive failure. In case average number of services is more than two or if conception rate on first service goes below 50% a veterinarian may be called to investigate.

Newly purchased bulls and calves should be examined thoroughly; their previous breeding record should be studied carefully. The most safest way is to purchase virgin heifers and bulls from disease free herds. It is advisable to quarantine newly purchased ones for some time to ascertain that their genital functions are normal and healthy.

If abortion occurs in cows they should be immediately isolated and veterinarian invited for investigations.

It is necessary to educate farmers concerning breeding hygiene, prophylaxis, detection of heats and maintenance of records.

It is also necessary to employ specialised veterinarian for regular periodical check up of the stock and for control of venereal infections and problems arising from low fertility, infertility and sterility.

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# Chapter 22

## Infectious Infertility

Infertility is classified according to the causal factors. These may be anatomical, functional or infectious. Infectious infertility is necessarily caused by infectious venereal diseases spread more rapidly by infected bulls in natural breeding or inadvertently through artificial insemination. One of the major factors responsible for the dissemination of coital or venereal infection is increased livestock movement from rural to urban areas and vice-a-versa for milk supply pockets and salvage purposes. (Fig. 81).

Reproductive failure in cattle due to infectious infertility results in lowered herd fertility rate and accounts for a considerable economic loss to dairy farmers. The infectious diseases causing infertility in cows and buffaloes may be grouped into Bacterial, Mycotic, Parasitic and Viral infections. Repeat breeding at regular or irregular intervals is a characteristic clinical feature of impaired fertility due to infections.

### Bacterial infections affecting bovine fertility

The causative factors of bacterial infections include Brucellosis, Vibriosis and Leptospirosis. These diseases are

mainly transmitted from infected cows to healthy ones by breeding bulls through coitus. Hence, they fall under the group of coital or venereal infections. Apart from the coital infections, Streptococci, Staphylococci or Corynebacterial infections of the tubular genitalia of cows are also encountered. The latter infections occur in post parturient cases when the cervix is open and hence are aptly termed 'Puerperal' infections.

### BRUCELLOSIS

#### Synonyms

Bangs disease, abortus epizooticus, contagious bovine abortion.

Brucellosis in cows and buffaloes is caused by 'Brucella abortus' and is essentially manifested at maturity. The disease commences in the form of bacteremia which continues in subacute and chronic form with organic changes of an inflammatory necrotic character causing abortion and infertility in females and pathological changes in the sexual organs of males.

#### Incidence

Brucellosis is prevalent in most of the countries of the world where intensive



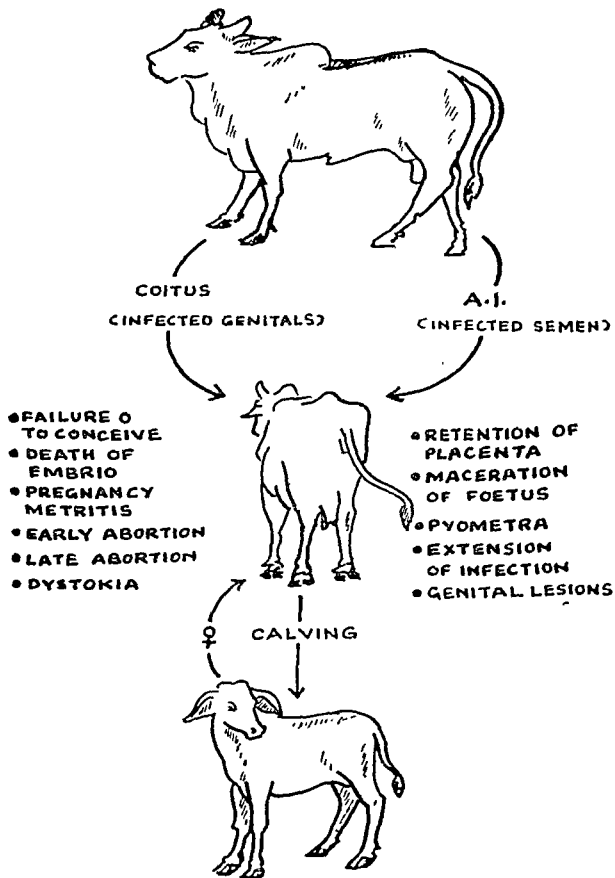


Fig 81. Factors involved in the spread of infections causing infertility.

cattle breeding is practiced. Scandinavia, Russia, Czechoslovakia and Finland have eradicated the disease. The incidence of brucellosis has been variously reported. In Great Britain according to Ritchie (1962) the incidence was 9.7% in 1950 which was reduced to 8% in 1956 and 2% in 1957-58. In Australia Brucellosis existed to the tune of 39.6% (in 1960), 8.8% in Canada, 26.1% in Germany, 2.8% in South Rhodesia. In Norway the infection reduced from 30% in 1956 to 0.6% in 1964 (Van Waveren 1965). In Brazil the positive reactors gave an incidence of 11.92% (Bernabe 1969). The incidence of 25% in 1944 was brought down in Denmark to 0.5% by 1957 (Thomsen 1957). In America the incidence has been reduced to 2.7%. Majority of the countries have undertaken the eradication programme.

### Historical background

Brucellosis in man was first discovered by David Bruce in 1887 from patients suffering from Malta fever. The chief host of the microorganism was proved to be present in goat's milk (Zammit 1905). It was in 1896 that Bernard Bang and Stribolt discovered the causal agent of abortion in bovines as the *Bacillus abortus* later on named as *Brucella abortus*.

### Economic importance

Brucellosis causes heavy economic losses of calf crop, infertility due to placentitis and reduction or total loss of milk yield following abortion or post-parturient metritis with delayed involution of uterus. There is depression in fertility as judged by conception rate. The secondary infections like bursitis, arthritis and tendovaginitis necessitate

slaughter. There is a decrease in the sale value of these animals. The routine breeding programme is markedly interfered on account of sterility in males due to orchitis, epididymitis or seminal vesiculitis. Enormous losses are annually incurred.

In addition to the above Brucellosis has public health importance since consumption of raw milk and other products as well as excretions from infected animals may transmit the disease to human.

### Etiology

*Brucella abortus* are small bacilli 1.2 microns long and 0.5 micron thick, non-motile and non-sporulating, sometimes occurring in cocci form. They are Gram-negative and stain easily with aniline dyes. Although the organisms are aerobic they require the presence of  $\text{CO}_2$  for optimum growth. The growth is profuse on agar or serum agar.

*Brucella* organisms cannot reproduce outside the body and therefore eradication can be easily accomplished. They can remain virulent in sterile uterine secretion in the interior of uterus and in dead foetus for months. They survive better in pelleted semen rather than in straw.

They are readily killed by sunlight, usual pasteurisation temperature and common disinfectants. They survive in cool wet places up to 3 months and in moist dung even up to 75 days. They are easily killed by compressed dung as in compost pits. They survive in chilled flesh and chilled milk for weeks or months but are not detected in over-ripe cheese. Air temperature of  $50^\circ\text{--}56^\circ\text{C}$  easily kills them in 2 hrs. or within  $\frac{1}{2}$  hr. in water bath.

### Routes of infection

In majority of the cases, infection occurs through the alimentary canal, through straw, fodder or drinking water contaminated by aborting cows. Less often infection may occur through the

genital tract during coitus or by infected semen. Infection through intact skin wounds or abrasions and wounds on teats is also reported. Infection also can be spread through conjunctiva. (Fig. 82).



Fig. 82. Transmission of Brucellosis.

### Period of incubation

Two types of incubation periods are observed in Brucellosis. The period from infection until the positive titre is reached is usually 30-45 days. The period from infection till abortion is variable depending upon dose, stage after calving and stage of pregnancy.

### Pathogenesis

The brucella organism on entering the host's body reach the regional lymph nodes (retro pharyngeal or mesenteric) leading to lymphadenitis and fibrosis. Later they enter blood stream causing bacteremia with rise in body temperature. Organisms are then conveyed to sites of predilection. In the female, these are the supramammary lymph node, udder, uterus, foetus and foetal membranes. In the male these are testes, epididymis, seminal vesicles and regional lymph nodes of reproductive tract.

### Symptoms

#### 1. IN FEMALES

In majority of affected cows the prominent symptom is abortion or premature birth if the endometrial lesions are severe but the calf may be carried to full term or be still born if the lesions are mild. Abortion may occur at any stage of pregnancy but most commonly between 6th to 8th month. Studies indicate that about 50% abort in the 7th month, 18% in 8th, 15% in the 6th and the rest in 3rd, 1st, 5th and 9th months.

In a clean herd brucellosis is recognised by an 'abortion storm' in which a large number of animals in various stages of pregnancy are involved. Highest rate of abortions occur in second calvers rather than in heifers. Infection then reaches its maximum peak between

13 years. According to Huddleson (1943) there is a tendency for the number of abortions to decrease in subsequent years. The animals acquire a sort of resistance and not many abortions occur between 3-7 years. This period of resistance is followed by another wave of abortions with a peak between 7-12 years and later subsides to a low level. Further continuation of the disease depends upon the size of the herd and type of replacement.

At the time of impending abortion the typical signs of abortion are noticed and the foetus is expelled with moderate signs of labour. The udder is swollen, painful and signs of mastitis are evident. Milk becomes salty.

Very often there is acute metritis with foetid, dirty grey or brownish red vaginal discharge in varying quantities. The uterine involution is delayed resulting in chronic metritis and infertility.

Placental lesions are not uniform. The maternal caruncles become necrotic and haemorrhagic with clumped villi usually covered with inflammatory exudate. The intercotyledonary areas are frequently thickened and opaque. The villi are thickened, distorted, covered with yellowish brown, gelatinous fluid, opaque and tough with characteristic leathery feel. Placentitis and adhesions usually accompany severe endometritis leading to retention of membranes.

#### FOETUS AND FOETAL MEMBRANES

The foetus and foetal membranes are oedematous. Foetal membranes may become 10 cm or so in thickness. Enteritis in foetus may be observed suggesting the swallowing of amniotic fluid. The body cavities contain sanguinous fluid. The abdominal contents are very turbid, lemon yellow in colour with flakes. Pneumonia in lungs is typical.

## II. IN MALES

The testes are often involved and acute orchitis sets in together with fever and loss of appetite. The testicles are swollen, painful, hot and doughy, the bull may refuse to mount on account of pain. The animal becomes sub-fertile or sterile due to thermal degeneration of seminal epithelium. In a certain number of cases increased libido may be observed possibly due to increased androgen production. In the initial stages semen contains high concentrations of spermatozoa but no motility. Later the leucocytes appear in the semen, flakes may be observed and it may be brick red in colour.

In chronic cases the pain subsides and the testicle becomes fibrotic, sequestrisation, hard and atrophic. Abscesses, sequestrisation and sloughing of the testicles may occur. Bleeding often occurs in the scrotal sac and the testicle undergoes pressure atrophy followed by necrosis.

Seminal vesicles are usually the first to be affected. Palpation of these on rectal examination is very painful. Acute stage is followed by chronic stage with considerable fibrotic induration.

### **Bovine infertility due to Brucellosis**

*Brucella abortus* itself is not likely to cause infertility as these organisms are not present in non-pregnant uterus and are already voided within two months following abortion or parturition. Delayed involution of uterus with retained afterbirth which is common in brucellosis results in puerperal metritis due to secondary bacterial infection. Subsequently, chronic endometritis and ovaro-bursal adhesions may result. Thus the main cause of infertility in brucello-

sis seems to be post-parturient puerperal infections. The transmission of Brucellosis as a coital infection from infected bulls to healthy susceptible cows by natural service has not been demonstrated in controlled experiments (Roberts, 1956). However, intra-uterine insemination with infected semen may transmit infection.

### **Diagnosis**

Brucellosis can be tentatively diagnosed by the history of abortion storms, localised symptoms of joints in male and female, signs of impending abortion, seminal vesiculitis and appearance of foetus and foetal membranes in female and orchitis in the male. However, the clinical diagnosis must be confirmed by bacteriological and serological examination.

Microscopic examination of the smears prepared from uterine and vaginal discharges, foetal membranes, placenta, effusions from foetal body cavities, abomasal contents, duodenum, lungs, spleen, bone marrow of foetus, abscesses from male genital tract-testis, epididymis, semen; milk from infected cow and fluid from hygroma, when stained with carbol fuchsin reveal the organisms. Cotyledon impression smears from aborted placenta, stained with saffron stain can also be recorded.

Inoculation of the above material in the guinea pigs confirms the disease. Pure cultures from the above material can be obtained on agar or serum agar with 10% CO<sub>2</sub> at 37°C.

Initially pooled milk sample from a herd is subjected to ABR test to detect presence of infection in the herd. If the test is positive all the animals in the herd are tested by quick blood agglutination test and sera from positive reac-

tors are further subjected to tube agglutination test for confirmation.

### Treatment

Brucellosis in animals is difficult to cure. Once the organisms are located in the uterus their multiplication cannot be prevented by medical treatment. Treatment is costly, lengthy and rarely successful. Symptomatic treatment should be carried out in an aborting animal and no curative treatment is done to the bull suffering from orchitis.

### Control measures

Isolation of all aborting and brucella positive parturient animals from a week prior to and a fortnight following parturition is essential. Disposal of aborted foetus, placenta, bedding soiled with uterine discharges, far away from the farm followed by thorough scrubbing, cleansing and disinfection of the cow byre with standard disinfectants are indicated. Positive reactors be segregated. Cows should be bred to known non-reacting bulls only, either naturally or by A.I. Eradication from a herd should be practiced when the disease becomes quiescent with an incidence of not more than 5 to 10% brucella positive reactors.

Calf-hood vaccination with cotton strain-19 has helped in considerable reduction of losses from Bang's disease. Strain-19 is also useful in protecting clean herds from adjacent sources of infection. This vaccine is to be used on female calves 6 to 9 months of age in which it brings about a febrile reaction and a low blood agglutination titre which slowly diminishes as the heifer reaches breeding age. Normally bull calves are not vaccinated as the vaccinal titre may result in rejection of an otherwise healthy bull from breeding pur-

poses or A.I. Vaccination of adult cows is accompanied by moderate pyrexia for 1 to 4 days with loss of appetite and reduction in milk yield. More systematic severe reactions sometimes occur with serious interference in milk yield in subsequent period of lactation. Several countries experienced difficulties due to the use of strain 19 adult vaccinations, the persistent titres interfering seriously with the determination of herd status and with tests for eradication purposes as methods of distinguishing vaccinal titres from others are not fully available. However, these hazards can be overcome by the use of vaccine — McEvan's 45/20 strain (McEvan, 1955). Duphvac N.A. vaccine can similarly be used. Herd can be considered free from brucella infection after every animal has passed two examinations of milk and blood (serum) with an interval of not less than six months. Brucellosis is communicable from animals to man. Drinking raw milk of carrier cows, buffaloes and goats is the common cause of infection. It is also a professional hazard (disease) of veterinarians, butchers and livestock workers who are likely to catch brucella infection through conjunctiva or skin. The veterinarian gets the infection while removing retained placenta or while handling cases of abortion or dystokia of infected animals. Symptoms appear after 1 to 3 weeks of incubation period and consist of undulant fever, headache, irritability and fatigue. Neuralgic symptoms, arthritis, orchitis and epididymitis may occur. Combination therapy of aureomycin and streptomycin gives better results. Preventive measures such as use of protective clothing for gynaecological examination, thorough disinfection of the rubber aprons, gloves and hands after handling infective material should be practised.

It is advisable to maintain a herd by its own progeny i.e. arrange to have own replacements. New purchases should be done from clean herds. The purchased animals should be isolated until proved negative. Home-bred bull or the one from clean herds should be used for service or for A.I. Grazing at common pastures should be avoided. Movement of cattle population should be restricted. Herd should be kept under regular blood testing programme if the incidence is high and under ABR testing if the incidence is low. Elimination of reservoirs and vectors of the disease (dogs, foxes etc.) should be the aim. Avoid feeding of contaminated feeds and drinking water. The first jets of milk should not be dropped on floor but be collected separately and destroyed. Carcasses of infected animals should be handled with care. Rail wagons and road trucks used for transport of animals should be thoroughly disinfected.

### VIBRIOSIS

Bovine genital vibriosis is a venereal disease of cattle caused by *Vibrio foetus* and characterised by infertility, delayed return to heat or irregular oestrous cycles, early embryonic death and abortion. Vibriosis is reported from most of the countries.

Genital vibriosis causes great individual and national economic losses due to abortion, repeat breeding, reduction in milk yield due to lengthened calving intervals in adult cows and delayed first calvings in heifers. Infected bulls may cause serious damage through wide-spread use either by natural service or A.I.

#### History and Incidence

McFadyen and Stockman (1909, 1913) reported cases of abortion in sheep and

cattle from Great Britain. Smith (1918, 1919 and 1923), Smith and Taylor (1919), Smith *et al.* (1920) and Smith and Orcutt (1927) published a series of papers dealing with the morphology, cultural characters and serology of *vibrio foetus* and its pathogenicity in cattle. Plastringe *et al.* (1947) observed that besides causing abortion, *Vibrio foetus* infection in cows was associated with infertility and that the breeding bull may get infected and spread the disease. Sjollem *et al.* (1949), Stegenga (1950) and Terpstra and Eisma (1951) reported a form of enzootic sterility in cattle in Holland caused by *Vibrio foetus*. Since then genital vibriosis has been generally recognised as a major cause of reduced breeding efficiency in cattle causing severe economic losses. The literature on bovine vibriosis is reviewed by Plastringe *et al.* (1951), Adler *et al.* (1952), Lawson and MacKinnon (1952), Plastringe *et al.* (1955), McEntee (1958) and Kaikini (1960).

Vibriosis has a world wide distribution. Its widespread prevalence in the U.S.A. was reported by Asdell (1952). It is also widely distributed in Europe. England, Germany, Sweden, Denmark, Holland and Norway have extensively reported infectious infertility due to *Vibrio foetus*.

Olson (1946) and Lagerlof and Banc (1951) showed that *vibrio foetus* was an active cause of bovine infertility in Sweden. Watson (1952) reported an outbreak of vibriosis in an A.I. station at Hongkong. Vibriosis was reported from Union of South Africa (Van Rensburg, 1954) and in Australia by Rickard-Bell (1954). Lawson (1954) and Hignett (1956) estimated that *vibrio foetus* infection alone or in combination with other factors causes about 30% of the herd infertility in United Kingdom.

Plastringe (1956) estimated that in the U.S.A. the loss from a reduced calf crop and milk yield was 137,734,000 dollars annually. Parnaik *et al* (1957, 1958) reported the presence of vibronic infection in cattle and buffaloes in Bombay State (India). Lundgren (1958) reported the presence of vibrio foetus from the breeding farms in Egypt (U.A.R.). Settergren and Soderlind (1966) during the course of their investigations on infectious infertility diseases in India isolated *V. foetus* from 2 bulls and 3 cows out of 272 preputial samples from bulls and 121 cervicovaginal mucus samples from cows. Of the 1413 cervico vaginal mucus samples from 6 different States in India, 125 proved positive for vibriosis by mucus agglutination test.

### Etiology

*V. foetus* is a gram negative bacterium, comma or S shaped rods varying in length from 1.5-5 microns and in breadth from 0.2 to 0.3 microns. It is motile. Commonly 3 serotypes of *V. foetus* are observed viz. *V. foetus venerealis* causing infertility in bovines but seldom sterility, *V. foetus intestinalis*, a natural inhabitant of intestines of cattle, sheep and pigs which sometimes invades pregnant uterus causing abortion, and *V. foetus bubulus* is saprophyte, occasionally pathogenic.

*V. foetus* can be grown in liquid media e.g. Thiol medium (Difco) which contains 0.3-0.5% agar and a reducing substance.

*V. foetus* is highly susceptible to light, drying and other adverse influences.

Infection is generally transmitted by coitus from cow to bull and vice versa. Sporadic cases of infection in young animals suggest transmission by other

routes. Infected semen diluted and stored at 5°C or even deep frozen, is capable of transmitting vibriosis to cows. Indirect transmission may occur between bulls at A.I. Centre through artificial vagina. Vibriosis is usually spread either by natural service with vibrio infected bulls or by artificial insemination with infected semen from carrier bulls. Plastringe *et al* (1955) tried to establish infection by (a) Vulvar exposure and (b) by swabbing the cervix with viable vibrio foetus organisms. The results were positive in the second method whereas it was variable in the first. Van Rensburg (1954) sounded the possibility of mechanical transmission by insect vectors, though the root of infection is confined to the genital tract. Lagerlof and Bane (1951) opined the possibility of bull to bull infection in artificial insemination centres due to poor hygienic and managerial conditions. McEntee *et al* (1954-56) also made similar observations. Adler (1957) presented evidence regarding the spread of vibriosis from one bull to another in artificial breeding studs.

### Susceptibility

*Vibrio foetus* is known to be pathogenic for cattle sheep, goats, pregnant guinea pigs, hamsters and possibly man. Mature cows and heifer's not previously exposed are susceptible.

### Symptoms

#### IN THE FEMALE

At the onset of disease, vaginal mucus appears red and vaginitis with increased secretion is observed. Terpstra (1953) observed a mild vaginitis of brief duration in some cows following service of an infected bull. The mucus may be clear, cloudy or purulent. This may last for 3-4 months. It causes low



grade or mild endometritis and salpingitis without any detectable changes on rectal examination. The animal repeats to oestrus due mostly to early embryonic death, on account of changes induced in the genital tract. The oestrous cycles become irregular. This type of infertility is characteristic of *V. foetus* (venerealis) infection. The infection in the female is usually self limiting.

In pregnant cows, early abortions due to *V. foetus* (intestinalis) upto the 4th month of gestation are common. In abortions occurring after the 5th month, foetal membranes may be retained. Abortion rate is usually low. According to Moore (1950), *V. foetus* infection is primarily a disease of foetal membranes and the foetus suffers secondarily from the gradually increasing interference with the placental circulation. Aborted foetus may show oedema of the subcutaneous tissue and haemorrhagic effusions in thoracic and abdominal cavities. Stomach content becomes thick, yellowish, grey coloured or cloudy with flaky exudate; normally it is colourless, viscid and translucent.

#### IN THE MALE

*Vibrio foetus* organisms are located in the prepuce of infected bulls. No pathogenic lesions seem to be produced in the prepuce and penis although there is permanent infection of the prepuce. Various workers have reported the recovery of *V. foetus* from preputial washings and semen of infected bulls. *V. foetus* infection does not seem to invade the male organs beyond external genitalia.

#### Diagnosis

Vibriosis may be suspected either due to early abortions or an increasing number of animals having irregular oestrous

cycles and repeat breeders in a herd. Less than 12% of infected cattle abort macroscopic foetuses, whereas, in the remaining cases early embryonic mortality results in which the only characteristic symptom is constant repeat breeding (Plastringe *et al*, 1951).

Diagnosis of Vibriosis on a herd basis can be done by testing the vaginal mucus of the cows and heifers of the entire suspected herd. The vaginal mucus gives a positive agglutination reaction to *V. foetus* antigen in about 40 to 80 days following exposure to the infection and the titre persists for about 7 months. Mucus samples may be taken from heifers which have been bred to suspected carrier bulls 2 months following the service.

Accurate diagnosis can be made either by isolating the organisms from suspected material collected from the infected animals or by means of specific serological tests. The former is applicable to both bulls and cows whereas the latter can be directly applied to the females only and indirectly in bulls after test mating of clean heifers.

#### I. BACTERIOLOGICAL EXAMINATION OF SUSPECTED MATERIAL IN THE COW AND HEIFER

Materials to be examined are aborted foetuses, placenta and mucus material from the vagina. Foetal stomach (abomasal) contents, lung tissue or amniotic fluid are also used. Suspensions (0.01 and 0.1 ml.) are inoculated into suitable culture media. Bovine blood agar or modified Plastringe medium may be used for primary isolation, whereas for maintenance of stock cultures, the bovine blood agar or peptone broth can be used. Cultures are incubated at 37°C in an atmosphere of 10-20% CO<sub>2</sub> for 3 days and then

examined for suspicious colonies. V foetus colonies are shiny, pale gray, semi translucent, flattened conical small and large ones are often seen on the same plate. Use of millipore filters (0.65 microns) prevents the contaminants.

A liquid mount is prepared and examined under a phase contrast microscope for *Vibrio* and *Trichomonads* and under a dark field microscope for *Leptospirae*. Freshly prepared smears stained with dilute aqueous fuchsin on microscopic examination will reveal the comma forms of V foetus but the most commonly seen are those which resemble the artist's flying seagull. Longer organisms with several convolutions are seen frequently.

Cultural methods are not very accurate in detecting V foetus infection in the cervico vaginal mucus, bull semen and preputial washings primarily because such material often contains saprophytic bacteria which overgrow the culture media.

Vaginal mucus for bacteriological examination should be collected at oestrus. Mucus taken between 7-21 days after first exposure to infection is also likely to be positive. This fact is utilised for the diagnosis of infection in bulls following the test mating of heifers.

## II. SEROLOGICAL EXAMINATION OF SUSPECTED MATERIAL

(i) *Blood serum agglutination test*  
Majority of cows aborting due to *Vibriosis* give significant titres at the time of abortion and for a fortnight thereafter. A negative titre at the time of abortion does not necessarily rule out V foetus as the causal factor. Persistence of significant titre in serum following vibronic abortion is usually of a short duration as compared to *Brucellosis*. Plastringe and Williams (1948) found

that 87% of 31 cows were positive at the time of abortion whereas of 27 that were retested about 3 months after abortion only 37% were positive.

This test has a very limited diagnostic value and therefore is not satisfactory in its practical application.

(ii) *Cervico Vaginal Mucus Agglutination test (CVMA)*  
This test is regarded as a very reliable test. Mucus agglutinins appear at a varying period after first exposure, 24 to 76 days and they may persist for 3 to 10 months and in some cases up to 30 months. Some cows may remain as reactors for life time. Samples should not be taken earlier than 5 weeks after infection. Specimens collected during oestrus usually give negative results though these animals may be positive at other stages of oestrous cycle. The agglutinin titre in chronic cases is reduced.

Stegenga and Terpstra (1949) first reported the presence of V foetus agglutinins in the vaginal mucus of cows in herds affected with *Enzootic sterility* in Holland. Later Terpstra and Eisma (1951) showed *vibriosis* to be the cause of *Enzootic sterility* and described the cervico vaginal mucus agglutination test and the pipette method of collecting the mucus. The pipette consists of a 50 cm long glass tube slightly bent at 10 cm from one end. It is inserted in the vagina and the mucus from near the cervix is drawn into the tube. The pipette is sealed after withdrawal and sent to the laboratory for examinations.

Szabo (1951) used a tampon placed in vagina to absorb mucus. Plastringe *et al* (1951-53) and Hughes (1953) used this method in USA. Boyd and Lagerlof (1954) compared both the pipette and tampon methods and showed that these are essentially alike.

Boyd (1955) in his extensive field investigation on bovine genital vibriosis covering 8,000 cattle in 105 Dairy herds in Sweden, used the tampon method for collection of vaginal mucus. Over 20,000 samples of vaginal mucus from near the cervix were collected and studied for this investigation. Tampon method seems to be ideal for routine vibronic detection work due to its simplicity in field and laboratory operations. The apparatus consists of a tube in which is placed the tampon with a string attached together with a plunger. A gauze 10 x 7 cm. and weighing approximately 1 gm. is folded to form a tampon about 8 cm. long, bound at one end by a 65 cm. fine strong string. Aluminium tube 45 cm. long with an outer diameter 1.5 cm. and inner diameter 1.3 cm. is used. The plunger is a rounded wooden or metal piece, 50 cm. long. The apparatus is packed in strong paper and maintained in dry heat at 120°C for one hour.

The tampon is inserted in the vagina in close proximity of the os uterus and retained there for 15 minutes where it absorbs the mucus and thereafter it is withdrawn. On withdrawal, it is put in a labelled wide mouth glass bottle and taken to the laboratory. Storing the samples upto 4 days at low temperature (+5°C) has no apparent deleterious effect.

In the laboratory 7 ml. physiological saline is added to each sample for extraction and left in the refrigerator (4°C) overnight. Next morning the liquid pressed out from the tampon is used as a test fluid for agglutination test with *V. foetus* antigen. Complete agglutination at any dilution is regarded as positive of vibriosis.

The result of the mucus agglutination test indicates that the individual

animal has been infected with *V. foetus* in the genital tract. It does not however indicate the presence or absence of active infection.

### In the male

In the bull, an accurate diagnosis of *V. foetus* infection can be made by the isolation of the organism from semen, preputial washings or both or from vaginal or uterine biopsies from heifers exposed by mating with infected bulls.

### DIRECT CULTURAL EXAMINATION

Semen is collected by properly sterilized artificial vagina. Preputial washings are taken after the semen ejaculate is collected. 75 ml. of broth adjusted to PH 7 or a medium containing Thioglycolate to which antibiotics are added to inhibit growth of saprophytes, is useful for obtaining the preputial washing. The broth solution is put in the preputial cavity, the preputial opening closed tightly, the entire penile region briskly and properly massaged for 10 to 15 times and the solution re-siphoned in the sterile bottle which is then sealed, labelled and sent to the laboratory for detailed cultural tests.

*V. foetus* colonies may be obtained from heavily contaminated semen samples and preputial washing by using ordinary blood agar plates containing 0.01% Alkylaryl sulphonate (Santomer) and 0.5% Acsulin.

### TEST MATING OF HEIFERS

In this method, *V. foetus* organisms are recovered from sexually matured maiden heifers mated to suspected bulls. Three to four days prior to the test mating, tissue biopsy samples from the cervix and posterior part of the uterus of the heifers are obtained by using Folmer Nielson Curette. These

are inoculated in the media for culture and should prove negative or the heifer is discarded. The selected heifer is then test mated to the bull or inseminated with semen to which the preputial washings have been added. Further biopsy samples are collected initially 2 to 4 days after mating or insemination and subsequently at 3 to 4 days intervals and inoculated in the cultural media. Negative cultures from 4 samples are regarded as an evidence of absence of V foetus infection and the heifer may be reused for testing another bull. Six negative biopsies within a period of 3 weeks indicate absence of V foetus infection in the bull.

### Control of genital vibriosis

Control of vibriosis is based on (i) its primary diagnosis and continued observations (ii) its transmission and course to prevent further spread (iii) therapeutic means to limit the number of infected animals and reduce the losses.

### Prevention

Prevention is better than cure and to achieve this strict hygienic precautions are necessary. Prevention is easily accomplished where artificial insemination with semen from bulls known to be free from vibrio foetus infection is practised.

Vibriosis can be prevented to a certain extent by purchase of bulls which are free from infection or those which never served before or the one which has served the virgin heifers only. The bull should be test mated to virgin heifers and introduced in the herd only when found negative or when proved negative by cultural tests.

Infected females should be isolated and it is desirable not to breed them

naturally. They may be bred by A I under strict hygienic precautions. Clean females may be bred naturally or by A I.

### Vaccination

Vaccination has been tried by many workers. Plastringer *et al* (1951), Beckenhauer (1967), Clark (1967), Lincoln and Tyout (1967). There is a vaccine of the killed cells of V foetus in mineral oil available for heifers. The vaccine is administered 2 to 3 months prior to maturity. There is limited information about vaccination of cows and bulls.

### Treatment

Treatment of the male and female stock with streptopenicillin or any other broad spectrum antibiotic is not very reliable and it is dangerous to maintain such animals in the herd, particularly where natural service is in vogue. Such animals should therefore, be culled.

## TRICHOMONIASIS

Trichomoniasis is an infectious venereal disease of cattle characterised by early abortion, pyometra and infertility and caused by a protozoan parasite, *Trichomonas foetus* transmitted to cows by infected bulls.

### History

The disease was first described by Kunstler in France in 1888 in a cow. Mazzanti (1900) is credited for its discovery in the genital tract of a slaughtered cow and a heifer. Emmerson (1932) first reported trichomonads in the fresh vaginal smears of two sterile cows from Pennsylvania, U.S.A.

## Incidence

Bovine genital trichomoniasis is reported from U.S.A., U.K. most of the continental countries including U.S.S.R., Scandinavia and India. In India for the first time, Ray *et al* (1956) detected the occurrence of a Trichomonad flagellate bearing a close resemblance to *Trichomonas foetus*. Das *et al* (1956) described the occurrence in Bengal of *Trichomonas* infection in cows suffering from severe vaginitis. Later in Bihar, Ishaque and Kuppuswami (1957) detected trichomonads indistinguishable from *Trichomonas foetus* in the vaginal discharge of a cow suffering from pyometra.

Morgan (1944) and Morgan and Winicky (1942), in a study of 560 and 1577 bovine uteri from abattoir recorded the incidence as 1.4% and 0.95% respectively.

Settergren and Soderlind (1966 a and b) during their investigations in India

on infectious infertility diseases in bovines, examined 293 samples from 253 bulls and 40 cows at Bombay and Bangalore for evidence of infection of *Trichomonas foetus*. Their findings were negative.

## Etiology

*Trichomonas foetus* is a flagellated protozoon having a pyriform or fusiform shape with three small flagella located at its cranial end and an undulating membrane extending along its body length terminating in a single caudal flagellum. It varies from 10-25 microns in length and 5-10 microns in width. In fresh preparations, the organism is actively motile with a jerking, twisting, spiral and erratic type of movement. It apparently multiplies by binary fission (Morgan, 1948).

## Pathogenesis

Trichomoniasis is essentially spread only venereally (Fig. 83). *Trichomonas*

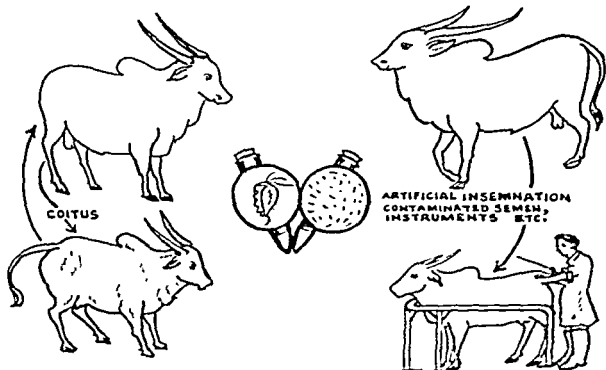


Fig. 83. Transmission of *Trichomonas Foetus*

foetus produces no pathogenic effects in the bull but is found as commensal in the prepuce. Hammond and Bartlett (1943) recorded that *T. foetus* organisms are much more numerous on the glans penis except galea glandis, although they are present all over the penile and preputial mucosa. Infection in the bull is usually permanent.

In the cow, the vagina is infected initially for 2 to 3 weeks without any well-marked vaginitis and *Trichomonads* invade the uterus during the period causing endometritis. The infection cannot prevent conception at initial exposure since the incubation period of *Trichomoniasis* is wider than the time required for the fertilised ovum to descend to the uterus. If conception occurs, the pregnancy is terminated at an early stage on account of endometritis. In other cases the gestation may continue for longer periods resulting either (1) in abortion most commonly at about 4 months or (2) the foetus may die in utero, become macerated resulting in pyometra. The stage at which pregnancy is terminated depends on the level of infection and the degree of specific resistance to infection. Spontaneous recovery occurs either at abortion, parturition or at the next oestrus period. Laing and Day (1917) observed that the *T. foetus* is capable of permanent colonisation in the uterus only during the time when a functional corpus luteum is present in the ovary viz. during dioestrus, pregnancy and pyometra.

## Symptoms

### IN FEMALES

The primary site of *T. foetus* infection is the bovine uterus. The cervix may also become involved. The uterine infections are characterised by low

grade endometritis, abortion or pyometra.

Symptoms of *trichomoniasis* in the cow vary from slight disturbances of the genital tract to those of severe infection. Normally within 24 hours following service by an infected bull, the vulva and vagina may become reddened and swollen accompanied by vaginal discharge. Between the 3rd and 9th day after service, the vagina becomes inflamed with increased mucopurulent vaginal discharge, which may be clear or cloudy containing white or yellow pus flakes. The discharge accumulates mostly in the fornix vaginae.

In acute vaginitis, there are haemorrhagic papules around the clitoris and the lateral walls of the vagina are inflamed with a marked proliferation of the surface lymphoid follicles. There is a characteristic raspy rough nodular surface of the vaginal mucosa. According to Fincher (1936) and Hopper (1941) this is a characteristic lesion of *trichomoniasis*. However, the nodules are very fine and may go undetected unless they are searched carefully on the vaginal wall just behind the external os. Low grade vaginal infections may clear up spontaneously and quite rarely they remain as carrier cows (Morgan, 1948). Infection of the uterus leads to a low grade endometritis accompanied by uterine, cervical and vaginal catarrh with intermittent or continuous discharge. The oestrous cycle may be irregular but majority of the cases come on heat at regular three weekly intervals. Such cases may conceive and calve normally resulting in spontaneous recovery. On the other hand, some cows may develop chronic purulent endometritis and pyometra which render them permanently sterile. Repeat breeding, delayed conception and vaginal dis-

charge are the clinical symptoms — in such cases. If conception takes place, early abortion may occur. Late abortions are very rare. Abortion may occur unnoticed following which the animal comes in heat, which is very often the first sign of trichomoniasis.

Pyometra develops due to early intra-uterine death of the foetus as a result of *T. foetus* infection with retention of corpus leutum and cervical seal of pregnancy. The foetus is not expelled but becomes macerated. There may be a slow escape of purulent discharge from the uterus when the animal is in recumbent position. The discharge is thin and grayish white in colour and almost odourless. The quantity of fluid may range to a few mililitre to 20 gallons with a pyometra persisting for over 2 years in some cases. If the case is treated in time and the fluid drained in a reasonable period, the cow may conceive later on. In most cases however, uterine mucosa is much damaged rendering the animal permanently sterile.

#### IN MALES

In bulls no symptoms are seen (Laing, 1957). Infected bulls may appear normal with or without lowered fertility depending upon whether they are being bred with susceptible or resistant cows. Ordinarily, the spermatogenesis, viability and fertility of the sperm is not retarded by trichomonad infection. Some workers have reported inflammation and a swelling of the prepuce with small nodules similar to the vaginitis described in the cows and accompanied by a mucopurulent discharge. This stage of acute inflammation usually subsides after a fortnight but the organisms may still be harboured in the preputial cavity, urethra, testicle and seminal vesicles and voided in the semen. In chronic

cases, bulls may develop orchitis and are unable to serve. In some trichomonad infected bulls, Breacher and Haffengartner (1933) reported severe balanitis and balanoposthitis.

#### Diagnosis

Trichomoniasis can be diagnosed by the demonstration of *Trichomonads* particularly during the second week following exposure to *T. foetus* infection. Likewise, *T. foetus* organisms may also be recovered from cases of open pyometra with purulent uterine discharge. Mucopurulent discharge and discharge containing flakes of pus invariably contain trichomonads. Material can be collected from the allantoic and amniotic sacs or foetal stomach in cases of fresh abortions. A positive diagnosis of bovine trichomoniasis essentially depends on the demonstration of living, motile, *T. foetus* flagellates either in the genital exudate of infected animals or fluid and stomach contents from infected foetuses.

*Trichomonads* can be recovered from the prepuce of the infected bull by collection of preputial washings or purulent discharge from the prepuce with a sterile cotton swab. However repeated examinations must be made before giving negative results. Test mating with maiden heifers followed by search for trichomonads in their vaginal discharge a fortnight later or mucus agglutination test be carried out in bulls. In either case, material for microscopic examination should be examined immediately after obtaining it or within a period of 4 hours after bringing it to a temperature of 37°C by incubation or use of a warm stage. Mucus for agglutination test should be obtained during dioestrus when it is scanty, rather thick and

opalescent In about 45 to 65 days following infection the agglutinins begin to develop in the vaginal mucus

### Treatment

Cows that have aborted early during the gestation period should be treated with the antibiotics — intra-uterine In positive females the treatment to the genital tract is on the same lines as that advocated for pyometra The use of broad spectrum antibiotics is indicated However the results are uncertain The affected animals may act as carriers for indefinite period If not culled these animals may be segregated treated and bred only by AI

The affected bulls may be treated with broad spectrum antibiotics infused through the prepuce Only bulls of exceptional value should be treated and reused only after they are proved negative to strict diagnostic tests

### Prevention and control

As Trichomoniasis is spread venereally it is essential that all breeding bulls should be subjected to vigorous andrological examination periodically and certified free from coital infections including trichomonad infection In case of AI bull this should be scrupulously observed to avoid any possible dissemination of infection through an infected or carrier bull Due to the variations in the production of agglutinins by infected cows mucus agglutination test is applicable only on a herd basis

Premium bulls pose a special problem as it is impossible to control the various types of cows presented for service by such bulls Microscopic examination of preputial material for the presence of the flagellates combined with further cultural examination is recommended

Use of separate sterilised artificial vagina for collection of semen for each bull is recommended Effective control can be established by the following methods

- 1 Elimination of infected bulls from the herd
- 2 Breeding in large herds must be done by artificial insemination using semen from clean bulls
- 3 Clean bulls must not be used for natural service or any cow or heifer ever bred to an infected bull
- 4 Good breeding performance in cows is not a definite evidence of freedom from trichomonad infection
- 5 Maintenance of accurate service registers for bulls and cows
- 6 Examine all mature females which have had any chance of infection or are suspected for any reason
- 7 Suspend all other services for 6 to 8 weeks
- 8 All infected bulls and cows may be segregated and culled
- 9 Examine all cows before service

### LEPTOSPIROSIS

Leptospirosis is caused by *Leptospira pomona*, *L. canicola*, *L. icterohaemorrhagica* and *L. hebdomadis* In cattle the disease is characterised by an acute febrile condition ( $103^{\circ}$ – $107^{\circ}$ F) with rapid lysis of red blood cells resulting in jaundice haemoglobinuria oedema and anaemia

### History

Leptospirosis was first recorded in Israel and later in Turkey Jungherr



(1944) first described it in U.S.A. at Connecticut.

Bovine leptospirosis has a wide distribution now in the United States and its importance has been increasingly recognised in many countries including Canada, Australia, New Zealand, Germany, Israel, Italy, Japan, Switzerland, Turkey and the U.S.S.R. According to Morse (1955), Leptospirosis is the fourth most important disease in the U.S.A. responsible for heavy losses to the farming community.

Sane and Deshpande (1965) during their investigations on abortions in Khillar cows, subjected sera samples from four aborted cows for detecting coital infections. The sera sample from one Khillar cow proved positive for *L. pomona* (+ 1:2400) infection. Settergren and Soderlind (1966) during their investigations on bovine infectious infertility in India, got 50 samples of sera tested by the agglutination lysis technique at the State Veterinary Medical Institute in Sweden. One of the samples from an aborted cow gave a titre of 1:2400 for *L. pomona*. This is probably the first record in India.

### **Etiology**

*Leptospira* is a spirochaete characterised by the extreme closeness of the turns, small size and curved or flexed extremities. It is 5-7 microns in length and 0.2 to 0.25 microns in width measured from crest to crest of the spiral (Alston and Broom, 1958). The organisms are easily destroyed outside animal body and seldom survive in free state for more than 6-48 hours.

### **Symptoms**

The incubation period in cattle is about 4 to 6 days in artificially produc-

ed disease and 10 to 50 days in naturally exposed females.

In cattle and swine, the affected animals show a rise in body temperature to 103° to 106°F. This is often accompanied by sudden drop of milk, anorexia and depression. In milder cases the animals may return to normal within few days but in severe forms of the disease a special type of mastitis is the sequelae. Milk production drops markedly and lactation ceases. The udder becomes soft, flacid and swollen; the milk secretion is pink, bloody or brownish in colour with large clots. In some cases haemoglobinuria and spiruria is observed which leads to icterus anaemia, subserous haemorrhages, nephritis (white spotted kidneys) and death. The mortality rate is usually high in younger stock upto 25% and in adults upto 5% (York, 1951); whereas Bryan (1954) reported mortality rate of 0.7% in 2817 affected adult cattle.

In milder cases, the pregnant cow aborts during convalescence. Abortions occur any time during the gestation period but usually during the last trimester (Mitchel, 1960). Whether the abortion occurs due to increased temperature and altered metabolism or whether the organism invades the uterus and kills the foetus is not definitely known. Since the abortion is not immediate after infection it is likely that the organism is destroyed and hence cannot be recovered from foetus or placenta (Roberts, 1956). Upto 25% of cows abort according to York (1951). Bryan (1954) and McTackch (1963) reported the abortion rate as 17% in U.S.A. and Australia respectively. Abortion may be followed by retention of after birth and consequent endometritis, however, this does not appear to be associated with serious failure to conceive

and most of the cattle recover after a sexual rest of 3 to 4 months.

Pigs are the chief reservoir of the organism (Bernstein and Baker, 1954). Wild life is considered to act as a natural host and eradication therefore poses a great problem.

### IN MALES

A bull shedding *Leptospira* organisms in urine would almost certainly be found to have them in the semen.

Leptospirosis is pathogenic to man, in whom *L. pomona* causes "swine herd's disease" whereas *L. ictero haemorrhagica* causes "Weil's disease" or "infectious hepatitis". The human symptoms are variable. But severe headache, with fever, conjunctivitis, muscular pains and encephalitic symptoms are reported most commonly.

### Diagnosis

Diagnosis is chiefly based on serological testing. The samples should be taken 1-2 weeks later since no titre is reached during febrile state. *L. pomona* has been the serotype most commonly implicated in bovine abortion. Diagnosis by recovery of *Leptospira* organisms from blood or urine with dark field microscopy or bacteriological cultivation is a difficult task. However, the isolation of *leptospira* organisms from aborted foetuses or its demonstration by histological examination of stained sections of kidneys has been reported from U.S.A., New Zealand and Denmark. Complement fixation test is less sensitive.

### Prevention and Treatment

Natural exposure develops solid immunity. The prophylactic measures include production of immunity by effective vaccination and prevention of

exposure to *leptospira* infection and isolation of infected animals. Cattle should be prevented from drinking water from stagnant pools, streams and separated from Swine as the latter are the important reservoirs of infection. The treatment is largely symptomatic and consists of parenteral use of broad spectrum antibiotics supported by blood transfusion in severe cases.

### VIRUSES AND INFERTILITY

During the past few years quite a large number of viruses have been reported to be associated with reproductive disorders. Some of them are fully recognised and have been classified as members of accepted virus group i.e.

- (1) *Herpes Virus group*: Infectious Bovine Rhinotracheitis - Infectious Pustular Vulvovaginitis (IBR-IPV) virus.
- (2) *Picornavirus group*: Enteric cytopathic Bovine orphan (ECBO) virus.
- (3) *Arbovirus group*: Rift Valley fever (RVF) and Wesselsbron (WB) viruses.
- (4) *Myxovirus group*: Bovine Virus Diarrhoea-Mucosal disease (BVD-MD) virus.
- (5) *Papovavirus group*: Bovine papilloma virus (BP).
- (6) *Unclassified viruses*.

### Herpes Virus group — IBR-IPV

This virus in addition to generalised infection, is also associated with infectious pustular vulvo-vaginitis, abortion and infertility in females. In bulls, it results in balanoposthitis and in newborn calves it is associated with fatal complications (Grieg *et al*, 1958; Kendrick, 1963; Vantoriumingen and Bartholomew, 1964).

## CLINICAL FEATURES AND SYMPTOMS

**Pustular Vulvo-Vaginitis:** The incubation period is very short and the disease is characterised by swollen and oedematous vulva and shows small pustules with yellow centre 1 to 2 mm in diameter with haemorrhagic borders. They may coalesce and lead to severe vaginal irritation. The lesions are confined to vulva and posterior vaginal mucosa accompanied by small quantities of yellow tenacious discharge. Animal may show temperature of 104° to 106°F and recovery usually supervenes in about 4 to 5 days. This condition may be confused with contagious granular vaginitis, however, in the latter there is no rise in body temperature, the lesions are small and firm and vaginal discharge is usually sticky and whitish yellow.

**Abortion:** Abortions may occur in pregnant cattle mostly in the third trimester of pregnancy after IBR-IPV vaccination. The animal is apparently healthy but often shows respiratory distress, conjunctivitis, vulvo-vaginitis, febrile reaction, abortion or still born foetuses and retention of foetal membranes. The foetal tissues and cotyledons appear pale and watery. The aborted foetuses are oedematous and show subcutaneous haemorrhages and serosanguinous fluid in the body cavities.

**Balanoposthitis:** With the rise in body temperature to 103° to 105°F after an incubation period of 1 to 3 days, the genital mucosa in the bull shows hyperaemia and petechial haemorrhages with specific pustules about 1 to 3 mm. in diameter, the pustules are smooth, raised and located on the penile mucosa. This is accompanied by swelling of penis and prepuce. A slight mucopurulent discharge is observed at the preputial orifice. The bull

is unable to serve due to the pain on account of ulcerated areas. Recovery often occurs within 10 to 14 days.

**Infertility:** The oestrous cycles become irregular. There is drop in conception rate. The affected animals show vaginitis with shiny, yellowish discharge which dries and adheres to the hairs of tail and the hairs at lower commissure. Animal shows recovery but is liable to be reinfected.

The affected bulls show enlargement of head and tail of epididymis together with orchitis. The seminal vesicles on rectal examination appear oedematous and enlarged. The semen examination reveals abnormal spermatozoa involving the shape of head in general and thickening of galeacapitis and narrowing of post nuclear cap in particular. The motility of spermatozoa decreases and pH of semen tends towards alkalinity.

## Diagnosis

The diagnosis of the viruses of this group is based upon the clinical symptoms and isolation of cell culture of virus from vaginal mucus. This must however be confirmed by serological tests and fluorescent antibody technique.

## Prevention and Treatment

Since the IBR-IPV virus can be transmitted by coitus, the affected animal should be isolated and natural service should be replaced by A.I.

There is no specific treatment and therapy should be symptomatic. The use of antibiotics to control secondary invaders and antihistaminic drugs may prove of some use.

## Pleorna Virus Group (ECBO virus)

Isolation of ECBO virus has been reported by Klein and Earley (1957) and

Straub (1965) The genital diseases and infertility of the male and female cattle are reported to be associated with ECBO viruses Florent *et al* (1962) reported the isolations of two viruses one from a bull with seminal vesiculitis and second from a bull with orchitis Because of their association with genital organs the presence of ulcers in the mouth and pneumonia, these viruses were designated as GUP Bouters *et al* (1964) further designated them as g8 and g10 respectively They were further characterised and classified by Bouters (1963) and Bogel *et al* (1963) as ECBO viruses Moll and Finlayson (1957) reported abortion in two cows when ECBO virus was recovered from one of the foetuses

#### CLINICAL FEATURES AND SYMPTOMS

##### In Females

*Catarrho vagino cervicitis (CVC)*  
The disease (CVC) is characterised by the presence of postcoital discharge in herds with history of infertility The vaginal mucosa shows varying degrees of congestion associated with that of cervix which becomes oedematous and protrude in the fornix The discharge yellowish in colour, gelatinous and tanaceous varies in quantity upto 100 ml This may be voided occasionally, smearing the tail and buttocks The course of the disease is variable from several days to three months being persistent in some cases and intermittent in others It is usually not accompanied by fever The conception rate in the herd is generally lowered and quite a large number repeat to services Prolonged anoestrus may also be observed

Moll and Davis (1959) reported abortions in cows from some herds after the respiratory involvement. The aborted

foetuses show serosanguineous, subcutaneous and intramuscular, oedema together with large quantities of fluids in serous cavities Abortions are common between five to seven months Still births and mummified foetuses are recorded

##### In Males

Florent (1962) stated that the disease is accompanied by respiratory and alimentary disorders G8 virus causes loss of appetite and stiff gait in bulls associated with conjunctivitis nasal discharge ulceration in the mouth and salivation (Florent 1962 and Bouters, 1963) The semen quality is seriously affected The spermatozoa show morphological changes particularly the degeneration of nuclei and mid piece loss of acrosome and exfoliation of cell membrane and presence of pyocytes Degeneration of germinal epithelium of testes is characteristic on histological examination Arrested spermatogenesis due to this virus was reported by Bouters (1964) A somewhat astonishing sign is the increased libido during acute phase manifested by frequent erections and ejaculations The seminal vesicles on rectal examination reveal the inflammatory condition

##### Source of Infection

The most common source of genital infection with these viruses is contamination of genital organs by the infected faeces In the females direct contamination of vulva from the dropping faeces or introduction of faeces during coitus may occur

##### Diagnosis

Diagnosis of ECBO viruses can only be done in the laboratory by the Serum Neutralization Test Haemagglutination

Inhibition Test may prove of some use. Isolation of virus in calf kidney cell cultures from the suspected material confirms the serological diagnosis.

### Prevention and treatment

As yet no attempts are made to prevent or to treat the ECBO virus infections. However it is worthwhile to expose the calves to ECBO virus infection so as to acquire natural immunity and thereby reduce the genital disease of matured cattle.

### ARBO Virus Group

In this group of ARBO viruses (Arthropod borne) are included two viruses viz. (i) Rift Valley Fever (RVF) and (2) Wessels Bron (WB), which are reported to be associated with abortions in cattle in Africa (Weis, 1957 a, b). These viruses are also associated with enzootic hepatitis and abortions in sheep and during these outbreaks, the pregnant cattle in association, may abort. The affected foetus is haemorrhagic and in the liver are seen the necrotic lesions. Karrer *et al* (1950) reported the transmission of natural infection by mosquito bite. A field rat (*Arvicanthus abyssinicus*) was considered to be a natural reservoir of RVF virus by Weinbren and Mason (1957). Both these viruses are pathogenic to sheep, cattle, horses, pigs, mice, rabbits, guinea pigs and man (Weis *et al*, 1956).

Diagnosis of RVF and WB virus is arrived at, by isolating the virus from the blood or tissues of the affected animals by inoculation of developing chick embryo, mice or cell cultures. Serum neutralization and complement fixation test are of great assistance in confirming the diagnosis.

Immunization with formalized vaccines prevents abortions in cattle (Weis, 1957).

### Myxo virus group

This group includes the BVD-MD viruses. The BVD was reported for the first time as a new disease (Olafson *et al*, 1946). The symptoms of which included stomatitis, gastro enteritis, laminitis and abortion. They reported abortions in 20 out of 35 pregnant heifers, within 10 days to three months. In an experimental infection with BVD virus, Baket *et al* (1954) produced abortion in one of the pregnant cows. In their investigations in 3 out of 39 herds, Robinson *et al* (1961) confirmed BVD as a cause of abortion, the diagnosis being based on clinical and serological findings.

Mucosal disease (MD) in young cattle clinically distinct from BVD was reported by Ramsey and Chivers (1953), which showed severe symptoms. Abortions and death of new born within 18 to 96 hours was very common (Schipper and Eveleth, 1957). However, they could not induce abortion experimentally. Experimental abortions with this virus was produced by Huch (1957) within 60 days.

The BVD and MD viruses are indistinguishable antigenically (Baker *et al*, 1954 and Gillespie *et al*, 1961).

Cattle, sheep and goats are susceptible to BVD-MD but the dogs, cats, mice, unweaned mice, hamsters, ferrets, pigeons, day old chicks or guinea pigs appear to be resistant. In pigs, the virus multiplies without producing any disease.

Diagnosis can be confirmed from clinical signs, haematological and pathological changes, serum neutralization

tests or agar gel diffusion tests. Inoculation of cell cultures may be useful for identification of the virus.

There is no specific treatment. The symptomatic treatment with antibiotics to check the secondary infection may be attempted. The non-pregnant cattle can be immunized with a modified live virus vaccine from rabbit or cell culture.

### PAPOVA Virus

Fibrio papillomas (warts) in the genital organs of cattle are reported to be caused by a member of this group (Iyengar, 1937, Frant, 1941, Lipinska and Krzvanowski, 1960). The lesions on the penile mucosa are in the form of single or multiple lesions. The preputial cavity and the vulva/vagina may also be involved. The bulls with papillomas often refuse to serve. Tumours are greyish white in colour and histologically consist of bundles of fibroblasts which are interwoven and covered by a thick layer of epithelial cells some times 10 to 50 cells thick. However, McIntee (1950) reported that no keratinization occurs. In the case of papilloma of skin, keratinization is present.

Papillomas can be prevented by the use of tissue vaccines prepared by the formalised suspension of warts (Pearson *et al*, 1958). The lesions can be treated either by surgical removal after the pudental block on the protruded penis or by the use of podophyllin or formalin solution, lactic acid or salicylic acid in an ointment base.

### Unclassified virus group

A number of viruses from the vaginal mucus membrane of cows suffering from vaginitis or vagino-cervicitis have been reported by McIntosh *et al* (1954), Miller (1955) and McClure (1957). They

produced the disease experimentally by intravaginal inoculation of the filtrate of the infected chick embryo material.

The clinical signs were similar to those of catarrhal vagino-cervicitis caused by ECBO viruses. McKercher and Kendrick (1958) stated that the disease is manifested in two forms viz (1) Vagino-cervicitis, lowering of herds fertility, irregular oestrous cycle, catarrhal endometritis and early embryonic death and (2) a syndrome causing infertility in both male and female cattle. Miller (1955) reported that the infection can reach the testicular parenchyma causing subacute orchitis and degeneration of germinal epithelium. Although the properties of these two viruses are similar, they are antigenically distinct.

Setka (1959) and Mensik (1960) described in details the viral orchitis in bulls from Czechoslovakia. One or both testicles are swollen and the acute stage may be followed by a chronic orchitis. The contractility of the cremaster muscle on the affected side is lessened. The rectal palpation reveals sensitiveness of the seminal vesicles and prostate. The fertility of the bull is reduced and the semen quality deteriorates. On microscopic examination a large number of abnormal spermatozoa are evident. The disease becomes chronic in few months and results in progressive fibrosis of seminiferous epithelium and atrophy of one or both testicles leading to temporary infertility. The virus is found in semen, testicles, discharges from female genital tract and ovarian cyst fluid.

The virus also affects cows producing purulent cervicovaginitis together with endometritis within 3 to 5 days after infection. Cystic degeneration of the ovaries results leading to infertility. The study of the causative virus is not

yet complete. However, it is known that this virus can be propagated on chorio-allantoic membranes.

From the vaginal biopsy of cattle affected with granular vaginitis, Yates *et al* (1957) isolated virus which was not related to IBR/IPV virus or ECBO virus.

Clinically, the granular lesions develop in the vulvo-vaginal epithelium. These lesions are more numerous in and around the clitoris associated with hyperaemia of the neighbouring tissues. Similar lesions may be observed on the penile mucosa of bulls. Microscopically the granular lesions consist of focal sub-epithelial lymphocytic infiltration. This may be accompanied by necrosis of the lining epithelium.

The role of this disease in fertility is not yet established.

The Miyagawanella infection may not have a direct effect on fertility. Some members of this group have a specific affinity for gravid uterus and are responsible for diseases of reproductive tract. Following the work of Stamp *et al* (1950) in Scotland on enzootic ovine abortion in sheep, outbreaks of abortions due to Rickettsia or members of Psittacosis Lympho-granuloma group of viruses has been reported in sheep in Bulgaria, France, Germany, Hungary, Japan, Sardinia and U.S.A., in cattle in Germany (Schoop and Kauker, 1956) and in goats in Germany (Staub, 1959).

Stors *et al* (1960) reported a similar disease causing widespread ovine abortion. Although this virus appears to be a member of PLGV group, it is not Miyagawanella bovis agent which causes sporadic bovine encephalo-myelitis. The pregnant cows show fever followed by abortion.

### Mycoplasmas (PPLO)

Mycoplasmas can often be recovered from bovine genital tract. They may penetrate the uterus some weeks after coitus by experimental infection of bulls. It appears that certain stress factors are necessary for production of lesions. They may induce balanoposthitis, arthritis and an acute reversible azoospermia occurring over a period of 2-3 weeks. During the azoospermia phase, the semen contains inflammatory cells, desquamated mother cells of the testicular epithelium and a few multinucleated cells.

### GENITAL TUBERCULOSIS

Tuberculosis of the genital tract in cattle and buffaloes is highly dangerous as the infection can easily be spread. The disease may produce infertility or sterility either by general breakdown in health as a result of extensive tuberculous lesions irrespective of involvement of the reproductive tract or by direct tuberculous infection of the genital organs. The lesions so produced may interfere with the production of ova or spermatozoa preventing conception or resulting in abortion.

Tuberculous infection is most frequently due to mammalian type (*Mycobacterium Tuberculosis bovis*) but occasionally abortions may occur in cattle due to avian type (*Mycobacterium avian*) of infection. The avian type does not cause progressive lesions of the uterus capable of preventing conception but there is every chance that the infected females may abort habitually (Plum, 1938).

### Incidence

The exact incidence of genital tuberculosis is not known. The disease is pre-

valent in all the domesticated animals and reported from most of the countries of the world. Dairy cattle and buffaloes especially are more predisposed on account of their isolation to stables and in ones which are confined to a limited grazing areas. The disease can occur in all species including man and is of importance from the stand point of public health as well as for its detrimental effects on animal production.

The incidence is much lower in cattle which are on the ranch all the year round. In beef cattle the degree of infection is much lower because of the open range conditions. It is difficult to assess the actual losses incurred every year but it is estimated that the infected animals loose 10 to 25% of their productive efficiency (Blood and Henderson, 1960). Spread of T.B. from animals to man through milk and air constitutes an important zoonotic problem. The tuberculosis testing scheme report published by the I.C.A.R., 1957 indicates that the incidence is higher in buffaloes (13.8%) than in cattle (4.7%) under Indian conditions.

Hutyrá *et al* (1938) observed 31% uterine tuberculosis out of the 580 cases of the generalised tuberculosis.

Reichter (1932) reported that breeding bull having orchitis which was mated to a number of cows resulted in the uterine tuberculosis with extensive peritoneal adhesions. Harmanssen (1925) reported incidence of 4.2% out of 12,000 cows tested in Sweden, of the positive ones 11.36% showed genital lesions. In an abattoir study in Denmark an incidence of 2% of uterine tuberculosis was recorded by Plum (1926). An incidence of 1.79% was recorded by him on a series of 831 foetal membranes. He studied 212 T.B. abortions and detected

tubercular bacilli in most of the specimens. He observed that most of these abortions occurred between 250 to 273 days of gestation period, which indicates that abortions usually occur in late stage of pregnancy. A survey report carried out in England on 82 cows with advanced tuberculosis showed that 23.75% were cases of genital tuberculosis (Burrow, 1937). In another group of 730 cows the percentage of genital tuberculosis was 5.8% (Cembrowitz, 1946). Hawden (1942) observed that out of 12,005 buffaloes carcasses (Bison bison) examined, 6450 (53.8%) showed lesions of tuberculosis in various organs but none in genitalia.

Sane *et al* (1958) recorded two cases of uterine tuberculosis in buffaloes. Deshpande *et al* (1966) recorded tubercular endometritis in a buffalo genitalia and they could detect the acid fast organisms in the tuberculous nodules. Similar observations have been made by Bhandari *et al* (1967) in a buffalo genitalia having tubercular endometritis and studied the consequent effects on cervix (Fig. 84).

### Epidemiology

Genital tuberculosis is most often secondary to primary focus in some other organ of the body. Uterine tuberculosis is most frequently haematogenous although it does also arise by direct extension from the peritoneum and oviducts. Tuberculosis of ovary, ovarian bursae and oviducts may have haematogenous origin or due to ascending and descending infection. However tuberculosis of vulva, vagina and uterus may be primary due to venereal infection. Primary tuberculosis of the female genital tract may also occur as a result of use of contaminated instruments.



Tuberculosis of the testicles is mostly secondary and most often haematogenous, however infection of the penis is probably always primary and venereal.

## Pathogenesis

### FEMALE GENITALIA

(1) *Ovary*: Ovarian tuberculosis is rather rare. It appears that the ovarian tissue is resistant to tuberculosis as compared to other parts of female genitalia. The ovaries may be found enlarged with small tubercles throughout their substance or they may be of normal size with granular surface. In advanced cases the ovaries may become adherent to the fallopian tubes and uterus and they become indistinguishable. When ovaries are completely surrounded by adhesions, follicles may become anovulatory, are prevented from ovulating, enlarge and become cystic.

Ovarian tuberculosis cannot be diagnosed clinically.

(2) *Ovarian bursae*: Tuberculosis of ovarian bursae is frequently associated with that of peritoneum, ovary and the oviducts leading to encapsulation.

(3) *Fallopian tubes*: Tuberculosis of fallopian tubes is not uncommon and is frequently associated with tuberculous peritoneum and uterus.

(i) In nodular tuberculous salpingitis, there are multiple nodules on the infundibulum and isthmus. The changes are not easily visible and can be diagnosed only on histological examination.

(ii) In caseous tuberculous salpingitis the changes are macroscopic. The fimbriated end is usually involved, which becomes thickened and occur in-between the wavy portions of the fallopian tube. The mucosa shows extensive

caseation. Tuberculosis of oviduct is usually bilateral and can be detected by rectal palpation. In bilateral affection the animal is mostly sterile.

(4) *Uterus*: Tubercular lesions may be found in the uterus without any changes in other parts of the genital tract but very frequently either the ovaries, fallopian tubes or less often the vagina may be found involved. Lesions in the uterus may be small and are usually localised around and on the cotyledons or there is every possibility that the lesions may extend throughout the substance of the uterus including the cervix. They may take the form of milary lesions on the surface of the mucous membrane. The lesions are evenly distributed but may be found localised in one horn or there may be larger nodules which may be ulcerated and which may extend deeper into the mucosa. In advanced cases the entire mucus and submucus tissues and sometimes the muscular coats are involved and the surface is seen extensively ulcerated. The lesions may be found to be caseous or calcified.

Tuberculous pyometra with gross enlargement of the uterus is not uncommon. There is foetal maceration in such cases. In tuberculous abortions, foetal cotyledons are necrotic and often covered by a thick exudate which often accumulates between them. Avian infection produces a mild type of tuberculous endometritis and placentitis and at times submucous abscesses are formed.

Clinically, in the bilateral uterine tuberculosis, the uterus is thrown into large transverse folds, palpable per rectum. In such cases, the enlargement is symmetrical and both the horns are alike in volume, form and consistency. This is accompanied with obstinate

uterine catarrh. However none of these warrants a positive diagnosis.

(5) *Cervix*: Tuberculosis of cervix is of rare occurrence. The growth may be of variable size and as such it is not so easy to confirm diagnosis just by rectal palpation. Moreover the bovine cervix changes its size to a considerable extent from calving to calving and at times the hypertrophy is so extensive that the diagnosis becomes difficult. The cervix of the buffalo however does not undergo such type of hypertrophy as in the cow. Bhandari *et al* (1966) observed in one specimen of buffalo cervix extreme hardness with kink formation and ectropion of the last cervical fold. Cervical mucosa did not show any discharge or nodules.

(6) *Gartner's ducts*: Tuberculosis of Gartner's ducts is frequently observed in association with or independent of uterine tuberculosis. It may be unilateral or bilateral. The ducts appear prominent, firm and nodular. The bead like nodules are arranged in rows and they may be intact or in some cases ulcerated. The presence of these nodules is regarded as a sign of uterine tuberculosis.

(7) *Vagina*: Tuberculosis of vagina is rare. However it may be associated with tubercular endometritis or cervicitis. Occasionally this may occur as primary infection through coitus. The neighbouring lymph glands are also involved and these are palpable. The lesions are chiefly in the form of ulcers of variable size.

(8) *Vulva*: Tuberculosis of vulva is a rare condition and may be associated with tuberculosis of vagina cervix and uterus. It may appear in the form of a cyst in the vulval lips which may contain a small amount of sero-purulent

matter. The affected labia is swollen and sclerotic. Vulvar tuberculosis is dangerous for the bulls.

(9) *Udder*: From public health point of view and the ease with which it easily spreads to the calves tubercular mastitis becomes an important problem. It is characterised by marked induration and hypertrophy of the udder, particularly the hind quarters. The supra-mammary lymph nodes are enlarged and form a diagnostic sign of tuberculosis of udder. Transmission through milk to human however can be prevented by pasteurisation of milk.

#### MALE GENITALIA

(1) *Testes*: Tuberculosis of testes is rather uncommon in cattle. So no primary lesions have been demonstrated. Tuberculosis of testes is generally secondary to tuberculosis in the other parts of the body. Two types may be distinguished microscopically:

- (i) *Disseminated miliary tuberculosis*: In this type miliary and larger tubercles undergoing caseation and calcification are seen scattered irregularly in the substance of the testes. The epididymis may however be free.
- (ii) *Chronic tuberculosis*: In this tuberculous changes in the testicle are characteristic and are in the form of delicate or coarser, caseating lines which radiate more or less closely from rete testis towards periphery. The epididymis is invariably involved.

*Tunica vaginalis*: Inflammation of the tunica vaginalis or periorchitis in bulls may or may not be associated with tuberculosis of testes. The condition

occurs due to the extension from peritoneal tuberculosis through the inguinal canal or direct extension from the testicle and epididymis. The lesions on the tunica vaginalis are in the form of pearl like structures varying in size and form.

The clinical diagnosis of tuberculous orchitis is rather difficult, since painless enlargement and hardening of testes could be due to other infections.

(2) *Epididymis*: Tuberculosis of epididymis is more frequent than that of testicles. The caput becomes greatly enlarged and firm. Miliary or large tubercles with central caseation and calcification may be observed in earlier areas whereas yellowish caseous spots undergoing softening may be seen at other places. The intervening tissue is markedly fibrosed and thickened.

(3) *Spermatic cord*: The inflammatory changes in the spermatic cord or funiculitis is quite common, but the tuberculous involvement is rare. The cord becomes greatly thickened due to the formation of typical epitheloid cell tubercles which undergo caseation and calcification.

(4) *Vas deferens and ampulla*: Tuberculosis of vas deferens is observed in cattle often associated with tuberculosis of epididymis. Vas deferens is converted into a thick, twisted cylindrical cord about 3 to 4 cm. in diameter. The cross section reveals a fibrous capsule surrounding a centrally located caseated mass. The ampulla may or may not be involved however its enlargement is palpable on rectal examination.

(5) *Seminal vesicles*: Tuberculosis of seminal vesicles is rare in cattle and unknown in other animals. The seminal vesicles are greatly enlarged and the cut surface reveals a centrally located dif-

fused caseated mass surrounded by a dense fibrous capsule. Lagerlof *et al* (1942) reported two cases of tuberculosis of seminal vesicles. One of the bulls had a hard and enlarged seminal vesicle and exhibited difficulties during micturition. The condition was diagnosed by rectal examination.

Nieberle and Cohrs (1967) stated that the vasdeferens remains unchanged in the tuberculosis of seminal vesicles.

(6) *Prostate*: Tuberculosis of prostate is of rare occurrence. In such cases, the prostate is greatly enlarged and is densely studded with caseous lesions of varying sizes.

(7) *Prepuce and Penis*: Tuberculosis of penis and prepuce is rare. The lesions in the form of nodules of linseed size, brownish in appearance are found in cutis, subcutis and glans. The surface is usually congested and eroded in places. The erosion is chiefly due to the destruction of the epithelium. The painful condition thus interferes with the introduction of the glans into the vulva during coitus. Small abscesses are located in the preputial wall and adhesions may be formed between the prepuce and the penis, preventing erection of the penis due to occlusion of the passage. In such cases bleeding may occur during coitus.

The penile lymph glands are invariably involved and are seen scattered from the sheath to sigmoid flexure. They are small and firm, not very painful and contain thick pus. On the presence of such lesions, a provisional diagnosis of tuberculosis can be made.

## Symptoms

### IN THE COW

Infertility is the primary symptom in the tuberculosis of the genital tract. In

the affected cases chronic vaginal discharge is usually present. There may be irregularity or absence of oestrus periods. In the well established cases of uterine tuberculosis the discharge is fairly copious and seen adhering to the ventral surface of the tail. The discharge is clear with flakes of whitish pus or wholly purulent and yellowish in colour. Vaginal examination will show the discharge flowing from cervix and accumulating in the anterior part of vagina. Characteristic tubercular lesions may be seen in the vagina or cervix or nodules palpated in the vulva. The vulval enlargement is conspicuous. Rectal examination may reveal on palpation the presence of adhesions in the region of the ovaries and perhaps also uterine enlargement in the shape of thickening of the uterine wall or there may be the presence of pyometra. In such cases, where there is extensive calcification of the uterine lesions their granular character can be felt on palpation. However the changes which are palpable per rectum by no means should be considered as a constant feature of tuberculosis of the genital tract.

In the affected females abortion may occur in late stage of pregnancy, most frequently during the last month but in certain cases it may also occur between 1st and 5th month (Nielsen and Plum, 1931).

#### IN THE BULL

Painless nodular swelling of the testicle or ulcers on the penile mucosa can be observed. There is palpable enlargement of the associated lymph glands. The quality of the spermatic fluid is deteriorated which may lead to impotency.

Generalised cases of tuberculosis are often associated with genital involvement.

#### Diagnosis

The tentative diagnosis in the females can be based on clinical findings such as bursal adhesions, obstinate uterine catarrh and enlargement of associated lymph glands, palpable gritty granular lesions in the uterus, nodular gartners canal and abortions in late stages. However the diagnosis must be confirmed by isolation of the organisms. Progressive emaciation is often associated with the disease and the symptoms become pronounced after calving.

The organisms may be detected from the smears of uterine discharge, exudate on or in between the cotyledons, foetal placenta or the foetus. It is not definite if the organisms can be detected at all times.

In case of the bull, the clinical findings in the genitalia viz. enlarged preputial lymph glands, nodular ulcerations on penis and uni or bilateral orchitis must be confirmed by isolation of tubercular bacilli in the semen, punctured samples from the testicle and material from penile ulcers.

The herd should be subjected to tuberculin test by which reactors can be screened.

#### Control

Regular testing of the herd against tuberculosis should be carried out.

No treatment is possible, however the positive reactors may be isolated, culled or slaughtered.

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# Chapter 23

## Cystic Ovarian Degeneration

Cystic ovarian degeneration or nymphomania is a multiglandular syndrome. It is characterised by the development of large follicles or cysts in the ovaries leading to the symptoms of either nymphomania with frequent, irregular or continuous oestrus or anoestrus or masculinisation with viraginity. It is also associated with infertility usually of a permanent nature.

The first description of nymphomania was given by Gurlt (1831). Earlier belief that infection is the cause for cystic ovarian degeneration was nullified as bacteriological examination demonstrated no relationship between cystic follicles and any recognisable infection of the reproductive organs (Quinlan, 1929; Webster, 1914). Eriksson (1939) and Lagerlof (1951) showed that there was a hereditary predisposition for cystic ovarian degeneration. Garm (1949) observed that the classical term nymphomania as used in Veterinary medicine actually comprises of distinct multiglandular syndromes viz. nymphomania and adrenal virilism.

In Sweden, Bane (1964) reported an incidence of 5.1%. Pålsson (1962) observed in Swedish cows an average incidence of 1.5% at an average age of 3.5 years. Hulthén (1968) stated that in Sweden there was a decrease in the in-

cidence from 7.6% in 1954 to 2.5% in 1967. In U.S.A., an incidence of 18.8% amongst 341 cows was reported by Casida and Chapman (1951). Bierschval (1966) reported the herd incidence to be 11% in the Holstein cows and 17% in the Guernseys. In Japan, Yamauchi and Inui (1954) reported an incidence of 9.7% of all infertile cows. In Russia, Pavuna (1964) found that the incidence of ovarian cysts in heifers was 7.49%. The incidence differed from breed to breed increasing from 2.94% for the Simmentals to 9.87% for Black Pieds.

### Incidence of Cystic ovarian degeneration in Indian Breeds of cattle

Luktuke and Arora (1967), reported an incidence of 2.21% follicular cyst and 2.60% luteal cyst in an Harijana herd. The incidence was highest during 3rd to 5th lactation (Table 27).

Table 27  
FREQUENCY OF OCCURRENCE OF CYSTS  
IN DIFFERENT LACTATIONS

Lactations	Follicular Cyst		Luteal Cyst	
	No. of cases	%	No. of cases	%
1 to 2	6	21.43	6	30.00
3 to 5	10	46.13	11	55.00
6 to 9	7	25.00	1	5.00
10 and above	2	7.14	2	10.00

The incidence of ovarian cysts was more in the right ovary than in the left (Table 28)

Table 28

## FREQUENCY DISTRIBUTION OF CYSTS

Type of Cysts	No of cases	Percentage incidence		
		Right	Left	Bilateral
Follicular cysts	28	60.71	39.29	—
Luteal cysts	20	75.00	25.00	—

When the cysts were manually ruptured through the rectal wall oestrus usually occurred within 4-6 days following treatment.

Of the animals treated for follicular and luteal cysts 56% and 61% respectively conceived as a result of service at the induced or first naturally occurring oestrus following treatment (Table 29).

An all India preliminary survey on bovine infertility was conducted by Luktuke 1952-53 when several organised breeding herds commercial dairy farms and cattle in rural areas were investigated. It was found that the incidence of cystic ovary was comparatively high in the milch breeds like Red Sindhi, Sahiwal, Tharparkar and in crossbred cows. The incidence was rare in village cattle.

Very little information of ovarian cysts is available for buffaloes. Luktuke and Arora (1970) recorded their observations for a period of 10 years in a Murrah herd that included on an average 67 buffalo cows and 14 heifers. They detected cysts in 42 buffalo cows and 7 buffalo heifers. Follicular cysts were recorded in 4 heifers and 28 buffaloes and luteal cysts in 3 heifers and 14 buffaloes. The incidence of follicular cyst was apparently high. The cysts were ruptured manually by palpation through the rectal wall the oestrus usually followed in 4-5 days. Out of 32 animals treated for follicular cysts 4 heifers and 16 buffaloes conceived. Similarly out of 17 treated for luteal cyst only one heifer and 6 buffaloes conceived. Most of the animals which had cyst in the ovaries were in the 1st to 5th lactation. The animals between 7 to 11 years of age had comparatively higher incidence of cystic ovarian degeneration (Table 30).

Table 30

## FREQUENCY DISTRIBUTION OF CYSTS IN VARIOUS AGE GROUPS

Age in years	Follicular cyst	Luteal cyst	Total
3-6	10	5	15
7-11	16	10	26
12-15	6	2	8
Total	32	17	49

Table 29

## CONCEPTIONS AT THE INDUCED AND SUBSEQUENT OESTRUSES

Oestruses	Follicular cysts		Luteal cysts	
	No. of Pregnancies	%	No. of Pregnancies	%
Induced	8	38.10	6	16.15
1st subsequent	6	28.57	5	38.46
2nd subsequent	5	23.01	2	15.39
3rd subsequent and more	2	9.52	—	—



They further observed that the incidence was high 181 days post partum.

### Etiology

The etiology and mechanism leading to the development of the cystic ovaries are not known. Many theories including infective factors, high protein diet and heredity were considered but it is accepted that hereditary predisposition plays a most important role.

Zschokke (1900) focussed attention on the hereditary nature of the disease. Eriksson (1939, 1954) concluded that heredity played an important role in its etiology. He recorded that normal dams produced 12% cystic daughters while as cystic dams produced 25.5% cystic daughters. Casida and Chapman (1951) estimated the heritability of the cystic ovarian condition in a herd of Holstein cows, as 0.13. Cystic ovaries occurred in 18.8% of the cows and 7% of the service periods. The proportion of the cows becoming cystic for the first time averaged 0.058 per service period. There was however, no evidence for association between butter fat content and this disorder. In heifers, the incidence of ovarian cyst was 3.4% and in cows in milk 6.8%. Garm (1949) showed that the cystic ovarian degeneration has a tendency for hereditary predisposition. Its incidence in cattle from 1 to 3 years of age was 18.9% (only 3 were virgin heifers); 54.2% between 4 to 6 years; 25.1% between 7 to 9 years and 6.8% over 10 years of age.

Roberts (1955) found that the incidence of cystic ovaries was highest (54.2%) in cows aged 5-6 years. With the increasing use of A.I. he found that the incidence of cystic ovarian condition also increased. This he ascribed partly to the greater use of A.I. which might have increased the transmission

by certain bulls. During mid-winter, there was a tendency for the incidence to increase in stabled cattle.

Henriksson (1956) stated that nymphomania is purely a hereditary disease which is sex linked and appeared in a homozygous form and is caused by a recessive gene with incomplete penetrance. He further stated that the disease was associated with high milk production.

In his investigation he found that the average frequency was 0.189 at a mean age of 4.49 years while the cumulative risk of the disease reached 0.58 at 11-12 years.

Garm (1949) stated that 45% of the nymphomaniac cows were excellent milk producers, 48% were fair producers and only 7% were poor producers.

Brodauf (1957) held the view that the disease is not hereditary but is rather a symptom of hormonal imbalance probably caused by defective environment. Palsson (1962) found significant differences in the disease incidence of cystic ovaries between groups of daughters of 5 bulls. The highest incidence, corrected to an age of 2.81 years, was 2.3% and lowest 0.33%. He observed similar tendency between progeny groups of other 7 bulls for which fewer records were available. The incidence was affected by season of calving, it being highest amongst July-September calvers.

Besides the work reported by Garm (1949), Casida and Chapman (1951), the hereditary predisposition for the inheritance of this condition has been reported by several workers as under.

### Genetic factors

Gonadal hypoplasia usually affected the right ovary in contrast to the cystic degeneration in the left ovary in cows

showing both the conditions Garm (1919) also observed similar association between cystic degeneration and high milk production.

Koch and Berger (1954) investigated a high incidence of cystic degeneration of ovaries in spotted Mountain Cattle in Austria and of the 119 cows examined 29 were affected. Out of these 28 were sired by one bull while 32 others of same bull were not affected. The dam of this sire was an outstanding milk producer and was treated for cystic ovaries. The 28 affected daughters produced on an average 462 kg. more milk and 0.39% higher butter fat than their dams. Though these daughter had good welset udders, they had a conformation of beef animals. This condition was attributed to a simple recessive mode of inheritance.

Gravert (1959) compared the reproductive efficiency of two bulls with 425 and 665 daughters respectively. The incidence of cystic ovaries in these two groups was 11.3% and 2.9%. The mean length of calving interval of cows that produced at least three calves in the two groups was 416 and 371 days respectively. The dam, the granddam and sisters of the first bull had longer calving intervals than those of the second bull. This was considered as suggestive of genetic involvement.

Varo examined 25,203 cows from two Finnish A.I. associations. 7.32% of Finnish Ayrshires and 5.62% of the Finnish cows had ovarian cysts. Marked differences were observed in daughters of different bulls and in about half the cases the cysts appeared to be of genetic origin. It was suggested that with examination of at least 100 daughters per sire, the carriers can be eliminated with a confidence of 90% (Svensk Husdjursskousel, 1961). Heida (1961)

studied 60 Freisian cows over three to five generations which initially originated from three sires and though he could not prove hereditary predisposition to nymphomania conclusively, indicated that genetic factors might be involved.

Maijala (1964) from a study of 5,000,000 service periods of Finnish and Swedish cows, reported that cystic ovarian condition was hereditary, specially in higher age groups.

### Mechanism of development of cystic ovaries

#### A. NYMPHOMANIA

Sonnenbrodt and Ranninger (1949) described cases of nymphomania in Lower Danube region and felt that it had a genetic basis. A sexlinked recessive gene was postulated which is expressed in females only. The disease manifests after the female has attained an age of five years or more and hence its control becomes complicated.

(i) *Hormonal imbalance*: In nymphomania, the primary lesion occurs in the anterior pituitary gland resulting in the disturbances in the relative secretion of LH and FSH. The proportion of LH and FSH is 3:1 in normal cows compared to 1:3 in nymphomaniac cows. The failure of the release mechanism or deficiency of lutenising hormone (LH) inhibits the normal development of corpus luteum. Garm (1919) observed that the persistence of the ovarian follicle as well as inadequate or nondevelopment of the luteal tissue indicates that the basophil cells in the hypophysis have lost their ability to produce LH. However, the FSH and ACTH production is increased. This explains the development of Graafian follicles and hypertrophy of the adrenal

cortex. Ovulation is inhibited due to inadequate production of LH by the pituitary gland. In majority of cases, the persistence of the follicles brings about prolonged production of oestrogen, which stimulates pituitary resulting in increased production of corticotropic hormones. Simultaneously oestrogens probably exert a divided action on the zona-glomerulosa of the adrenal glands. The secretion of deoxycorticosterone increases due to the cortical stimulation exerted by the oestrogens and the hypophysis. This results in the disturbances in the blood electrolytic balance of elevating — sodium ion in the blood serum and in the extra cellular fluids, which causes increased osmotic pressure with a corresponding retention of fluids and development of oedema.

Other hormonal factors might induce this syndrome e.g. a lack of progesterone in the mature graafian follicles to stimulate the release of LH., an imbalance between FSH and LH, increased production of LTH or growth promoting hormone (STH) causing high milk production levels, inhibition of the release mechanism of LH by the hypothalamus or possibly other effects on the anterior pituitary gland by thyroid and adrenal.

(ii) *Neural factors*: In nymphomania hypothalamus acts directly in relation with environmental conditions and neural systems and also in combination with hormones. Anoestrous condition observed in some cows may be produced by oestrogens through 'oestrous Block' of the nervous system. Hetzel (1940) postulated that the cyst formation was due to an increased osmotic pressure exerted by the follicular fluid causing degeneration of epithelium.

## B. ADRENAL VIRILISM

This syndrome might be the result of an excess production of androgenic substances of corticosuprarenal or ovarian origin. The adrenal glands which normally secrete small amounts of testosterone (male sex hormone) may also become involved resulting in increased secretion of testosterone, simultaneously, the cyst ceases to secrete oestrogen. This leads to complete anoestrus and the female may develop distinct masculine characteristics such as gain in weight, thickening of the neck, developing male voice and steer like appearance.

## Symptoms

On the basis of clinical symptoms Garm (1949) classified the nymphomaniac cows into four groups as under:

Group-I — Nymphomania with permanent heat or periods of intense heat at frequent intervals.

Group-II — Nymphomania with normal heat at regular intervals.

Group-III — Nymphomania with mild symptoms of heat at infrequent intervals.

Group-IV — Nymphomania with ceased heat.

The occurrence of heat at irregular intervals is characteristic of early stages but later it becomes continuous. The nymphomaniac cow may remain in oestrus continuously for 4 to 5 days every week or show oestrus every 3rd to 4th day. The behaviour of the cow is anomalous presenting bull like features in the earlier continuous oestrous phase, which passes into anoestrous phase.

Holy (1964) studied arborization pattern of cervical mucus and appearance

of cervix vaginal mucosa and mucus in 102 cows with cystic ovaries. In 44 cases he found that ovarian cysts were accompanied by increased sex desire (only five had typical nymphomania) and 58 by prolonged periods of anoestrus. Arborization pattern indicative of high oestrus activity was present in 94 irrespective of outward symptoms. Colour of the vaginal mucosa and cervix and consistency of mucus apparently indicated high follicular activity. The pH of vaginal secretions was alkaline.

### 1 Nymphomania with permanent heat

*Changes in the body conformation* In majority of the cases of nymphomania relaxation of the pelvic ligaments produces sinking of the pelvis and elevation of the root of the tail. This elevation of the root of the tail is called the Sterility hump because it tends to persist after recovery. In many cases even after recovery and subsequent conception the ligaments fail to regain their tone. The ischial tuberosities also appear to be elevated and there is a ventral dropping of the lumbosacral articulation. This tipping of the pelvis may be responsible for the unsteady gait which may lead to injury. When the cystic ovarian degeneration condition prolongs the cow may put on more weight and develop a thick neck and herd simulating a steer.

*Clinical symptoms* These animals show continuous intense heat mounting other cows in the herd or will stand to get mounted. They mount even on inanimate objects. They are nervous restless and bellow frequently. They lose weight because of frequent movement on the pasture and are very aggressive.

The vulva may increase in size, relaxed, and swollen. Copious, tenacious

and opaque discharge may appear at the vulva. The mucus is whitish grey in colour appearing as a mucopurulent discharge. The os cervix is usually enlarged, dilated and relaxed. The uterus is oedematous and atonic. Its walls are flaccid and on palpation seldom show tonicity or become erect or turgid as it occurs in a normal uterus especially prior to or during oestrus. Rectal palpation also indicates the relaxation of the pelvic ligaments. Milk yield decreases and according to some authors it is salty and bitter in taste. In some cases vaginal musculature is so relaxed that posterior portion may get prolapsed.

Cysts varying in number (1 to 4) and in size (1.5 cm to 6 cm in diameter) are thin walled usually peripheral in location and may burst readily with pressure (Fig 85). Repeated examinations and the history of the case are helpful in differentiating between a cyst and a follicle.

The distribution of the cysts in the ovaries is tabulated (Table 31).

Table 31  
DISTRIBUTION OF THE CYSTS  
IN THE OVARIES

Author	Percentage incidence			
	Total	Right	Left	Bilateral
Danielius				
1914	100	16	12	12
Garm 1919	100	31	19	50
Vandeplassche,				
1951	100	65	35	—
Roberts, 1955	100	33	23	14
Pavuna 1964	—	10 17	23 02	35 71

It is observed from the table that the right ovary is involved more as compared to the left. Investigations of Garm (1919) revealed that multiple cysts are

more common than single ones and further showed that large single cysts were formed by the union of several cysts. Garm found that the membrana granulosa was thinning out and the theca interna degenerated in the later stages though the basal portions of the cysts were sometimes lined with a very thin layer of luteal tissue. Corpora lutea are very seldom observed in nymphomaniac cows. The fluid in the cyst is usually clear but sometimes of amber colour. In many cases there is absence of ovum and granulosa cells. Some granulosa cells may be found at the basal portion in the cyst which may get luteinised.

In respect of the contents of the cysts, Lesbouyries (1944) demonstrated moderate oestrogen content in the follicular fluid samples. Yamauchi and Inui (1954) in the biological assays found oestrogen 833 to 4000 I.U./litre in a cyst in each nymphomaniac cow as compared to an average of 432 I.U. in the Graafian follicles of oestrus in 10 normal controls. Almost half the cysts from the nymphomaniac cows did not yield oestrogen titres. The toxicity of oestrogen depends not only on the levels of circulatory oestrogen but also on the continuity of its action. Large, mucus-secreting cells make up the superficial epithelium during proestrus and estrus. The layers of cells in this area are reduced from 3 or 4 during proestrus to 1 or 2 during estrus and are increased to 3 or 4 again by 2 days postestrus. At 2 days postestrus the cells are cuboidal and ragged and at 8 to 11 days postestrus the epithelium is vacuolated and appears degenerated in character. Desquamation has not been observed in this portion of the vagina.

The vaginal epithelium near the urethra is irregular in depth throughout the cycle, varying from 4 to 33 cell layers. The superficial cells are polyhedral rather than squamous in type. During estrus the average epithelial cell height increases to as much as 54 as compared to the 46 found during proestrus. The stratum germinativum becomes more pronounced and the nuclei have their long axes perpendicular to the membrana propria. Lymphocytes and leucocytes are present in all epithelial layers.

Uterine mucosa undergoes considerable morphological changes in nymphomania. Mucus is not usually present but occasionally upto 100 ml. of it can be recovered from uterus. There is marked hyperplasia of the endometrium with the cystic dilatation of uterine glands. Garm (1949) rarely encountered endometritis but emphasised that the intra-uterine medium and the possibility of infective agents entering the uterine cavity in certain types of nymphomania favour the development of endometritis. In nymphomania, the uterus is large and flabby with oedematous walls. The cervical canal is wide and open. The uterine mucosa is thick and oedematous, but, the gross appearance is otherwise normal. The mucosa is smooth, moist, semitransparent and grayish pink in colour. Garm (1949) observed on the uterine mucosa numerous pin head sized, greyish, semi transparent rounded elevations with a thin transparent capsule. In extreme cases the dilatation of numerous cysts gives an appearance of "Swiss Cheese".

The proportion of the total pituitary weight and volume represented by the pars anterior (Pars principalis, pars distalis) is somewhat larger in the nymphomaniac cows than in the endocrinally

normal cows The anterior lobe is enlarged due partially to stromal hyperplasia and oedema and partially to cellular hypertrophy The gonadotropin producing basophils normally degranulate and release their hormone in the first hours of oestrus If there is no release of hormone, the gonadotropin producing cells remain fully granulated The degranulation is delayed and is completed in case of luteal cysts Garm (1949) indicated that changes in the pituitary gland to a major extent are due to over exertion during the high lactation period and that they are prior to the changes in the ovaries and adrenals However he suggested that the prolonged ovarian production of oestrogen in most types of nymphomania contribute to the changes in the hypophysis

In nymphomania there is increase of weight of adrenals mainly due to hypertrophy of the adenal cortex In majority of cases the zona glomerulosa is weaker than in normal ones Garm (1949) suggested that the cortical hypertrophy is due to prolonged influence of oestrogens The zona glomerulosa produces mineralo cortico steroids which regulates the electrolyte and fluid balance of the animal body He concluded that mineralo cortico-steroids are concerned in the cystic development of follicles relaxation of pelvic ligaments oedema in the genital organs, atony of uterus and salty taste of the milk produced

No regular changes of the thyroid, parathyroid and epiphysis could be observed neither regarding the gross nor the microscopic appearance (Garm, 1949) The results of the chemical analysis by Garm (1949) indicated that the total thyroid iodine content in nymphomania vary within normal limits

## 2 Nymphomania with normal heats at regular interval

Cows under this group exhibit clinical symptoms lesser in intensity than in group I Garm (1949) has also reported nymphomaniac cases with normal heat symptoms and inter oestral period under this group These animals have sunken pelvic ligaments and did not become pregnant

## 3 Nymphomania with mild symptoms of heat at infrequent internals

The clinical symptoms are mild The animals show weak intensity of heat which may appear at short or long intervals Animals do not hold to services The ovaries usually have either large single or small multiple cysts

## 4 Nymphomania with ceased heat (prolonged anoestrus)

The cows in this group are fat and indolent No oestrus symptoms are exhibited and they do not become pregnant Adrenals are considerably enlarged The output of neutral steroids in the urine is less than that in the normal Roberts (1956) has reported that 25 per cent of the cystic ovary cases observed by him fall in this group Some cows in this group may fail to exhibit oestrus after evincing normal heat The owners may sometime believe that the cow is pregnant A large number of cows may have relaxed pelvic ligaments The uterus is not always large as is found in animals of the above groups and sometimes it can be much smaller as well But the ovaries contain single or multiple cysts Mucus discharge at the os is scanty and the animals do not stand to get mounted by bulls even though they appear to be in heat

In anoestrus type, uterus is usually small and shows atrophic changes. In these cases as well, there is hyperplasia of endometrium resulting in cystic dilation which occurs as a result of increased oestrogen activity from ovarian cysts. In extreme cases, these glands dilate extensively resulting in mucometra or hydrometra because of marked atrophy of uterine wall.

### Treatment

Approach to the treatment of cystic ovarian condition in the bovine is very variable since it is dependent on the accurate diagnosis of the structural changes involved. Effective treatment can only be done to a single cyst which is thin walled and situated on the periphery of the ovary. In case such a cyst is palpated per rectum it can be ruptured manually by digital pressure. Thereafter the cow is expected to come on heat in about a week or so. This heat should be carefully observed and animal served in good time.

On rupture of the simple cyst the animal will show normal heat, follicular development, ovulation and conception. In case it is found that the heat is prolonged it is quite likely that there

attempt repeated rupture of the follicular cysts. In case of multiple cysts it is likely that considerable damage might occur to the ovarian tissue due to digital pressure used during rupture and it is therefore not advisable to adopt this line of treatment. Moreover, in case of multiple cysts chances of conception are practically remote on account of damage to the myometrium and endometrium and the degenerative changes that are in progress.

In cases where there is intense heat at frequent intervals it is a very clear indication of nymphomania and in such cases it is inadvisable to undertake any line of treatment considering that hereditary factors are involved.

Cows which apparently appear normal but which may show no symptoms of heat from cycle to cycle, may show follicular cyst or cysts in their ovaries with typical histopathological changes in the myometrium and endometrium. Hormone therapy has given inconclusive results and there being hereditary tendency it is not advisable to treat such cows.

Similar approach also holds good in case of cows which show regular heats, but which fail to conceive. Repeated

tations of surgical removal and recurrence of the condition (Sane and Diwan, 1977).

### Hormonal treatment

Various workers have used hormones for the treatment of cystic ovaries. Fiorello (1952) claims good results in 10 out of 25 cows by giving intra muscular injection of 10,000 IU of gonadotropin. Vandeplasseche (1951) and Paredis (1953) injected 500 IU PU directly into the follicular cyst through the vaginal wall and this resulted in permanent cure of nymphomania in 25 out of 36 cows. Grigor (1955) treated 9 cases out of 11 with intra muscular injection of 10,000 IU of chorionic gonadotropins (Antuitrin S).

A recovery rate of 37.2% in 188 cases was recorded by Roberts (1956) after manual rupture of cysts, whereas Vander Plassche (1951) claimed 42.5% out of 54 cows, within one or two months. Of the 21 — gestation periods of these cows, 5 ended in abortion and one had twins. Clapp (1934) reported that out of 21 cystic ovaries in cows; 9 (37.5%) conceived twins after treatment. Due to this high incidence of twinning, service may be deferred till second oestrus.

Number of reports show that oestrogens and testosterone used in cystic ovaries have shown unsuccessful results. Results with LH and chorionic gonadotropin are also uncertain and inconclusive. With any line of treatment, hereditary tendency for this condition has to be borne in mind.

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# Chapter 24

## Infertility in the Mare

The three chief reasons for infertility in the mare are:

- (1) Short duration of breeding season.
- (2) Genital infection.
- (3) Abnormal oestrous cycles.

Irregularities of oestrous cycle constitutes one of the most important cause of infertility. It is not possible to do anything about the length of the breeding season, especially in thorough-breds whose age is dated from January first of the year they are foaled. Genital infection can be controlled to a great extent by proper hygienic conditions and prompt treatment. Day (1939) stated that stallions with low fertility and mares having rare genital abnormalities contribute towards infertility. He estimated average fertility in draft mares as 59% in through bred under better management practices as 66% and 90% in wild ponies that run free with stallion. The average conception rate in mares is usually lower than in other domestic animals. However, under optimum conditions of management it may reach 80-85%. In majority of the breeding farms the conception rate is about 60-75%. Most of the causes of infertility in mares are usually of transi-

ent nature. In the continental countries on well managed stud farms mares are fed on adequate ration of green roughages with some legume preferably clover. Legumes have a higher content of minerals than Timothy. Pasture grazing supplies roughage in the best form. Grain feeding should be adequate to maintain condition and supply the energy and protein requirements. Fresh water and iodized salts should be made available at all times.

The breeding season for thorough bred mares is from 15th February to 31st July. It is therefore necessary to see that all mares to be bred, should have excellent condition at the beginning of the season. A constant attention is necessary on barren mares to see that they are examined from time to time and treated, if necessary. Apparently pregnant mares should be examined during the fall months to make sure that the early abortions, which are quite common between 25 to 90 days, did not occur. Pregnant mares should be protected against likely abortions.

### Irregularities of oestrous cycle

Maintenance of upto date breeding records are most essential to ascertain irregularities of oestrous cycle.

Teasing of the barren mare should begin about a month before the commencement of breeding season. Such a practice builds confidence in the mares in respect of the teaser or the stallion and they are less likely to be frightened during services. The behaviour of the mare and mare's reaction towards teaser should closely be observed for signs of approaching oestrus. Teasing should be commenced on the 3rd or 4th day of foaling. Errington (1951) observed that certain mares fail to show oestrus to certain teasers. Pregnant mares may occasionally show heat for a short duration. Fatty mares may be often phlegmatic towards teaser and fail to come in oestrus.

All barren and foaled mares should be teased daily or every other day until they are bred. Teasing should be continued until the end of oestrus. Mare still in heat 3 to 4 days after service, should be re-served in the same heat. During the next two weeks teasing is necessary only every 3rd or 4th day. Two weeks after breeding, teasing is done daily or on the alternate days. If heat does not recur, teasing every 3rd to 4th day may be continued for several weeks more and then mares should be examined for pregnancy. A certain number of barren and foaled mares may show marked irregularities in the oestrous cycle. Majority of foaled mares, however, have regular oestrous cycles. Mares which are not bred on foal heat may develop anoestrus for long periods. This may occur in mares which are in poor condition and in those which are heavy in milk. Caslick's (1937) reported that certain mares with irregular oestrous cycle ovulate regularly but fail to show heat at every cycle. Crowhurst and Caslick (1946) observed that 80% of foaled anoestrous mares returned to

oestrus spontaneously without external stimulation in time to be bred before the end of breeding season and 40.4% conceived.

Oestrous cycle irregularities were divided by Caslick into four types:

### 1. LONG PERIOD OF OESTRUS

This type includes 75% of the barren mares. Heat period may last on an average from 10-20 days or in extreme cases even upto 80 days. During this period the mare will accept the stallion at any time. Such abnormal cycles are usually observed in the early breeding season and as the season progresses, the periods of estrus become shorter and cycles become normal. Breeding should be delayed until the cycles become normal. This condition may simulate nymphomania in cattle but no cysts can be detected in the ovary.

### 2. PROLONGED DIESTRUS PERIODS

This group includes about 15% barren mares. When oestrus is exhibited it is true period. Ovulation appears to be regular and oestrus is not exhibited at every ovulation. This condition simulates suboestrus or silent heat in cattle. The barren mares do not conceive as readily as those in group one. After foaling about 50% of them have normal oestrous cycles and the other half continue to maintain the irregular type. These mares are bred when they show good oestrus.

### 3. IRREGULAR PERIODS OF OESTRUS

This third group of barren mares is characterized by frequent prolonged periods of oestrus over the days in diestrus. This class may include mares with split oestrus condition. In this condition mares are in heat for one or more days, then out of oestrus for one to two days and again back to oestrus

for one or more days. These mares are usually good breeders.

#### 1 NO OESTRUM

In this class manifestation of sexual desire is absent but ovulation is usually regular. The teaser is of no use for this class. Mares in this group are usually poor breeders.

Caslick stated that during breeding season abnormalities in ovulation are rare. Ovulation usually occurs at regular intervals and is not influenced by irregularities of oestrous cycles. The various manifestations of irregular cycles appear to be inherited; and pregnant mares may occasionally show short oestrus of about a day late in the breeding season. Mares exhibiting oestrus during gestation period should be examined to find that abortion has not occurred. Coitus may induce abortion.

#### Anoestrus

This may be due to poor condition, inanition or starvation. It may also be met with in fatty mares, foaled and barren mares. True anoestrus is characterized by the failure to exhibit oestrus towards the teaser and presence of small hard fibrous, inactive ovaries without follicular development. The barren mares are usually in good condition. Treatment of such a condition with oestrogens and gonadotropins has not proved successful.

Treatment of such mares in anoestrous condition is reported to be successful by administration of 500 ml. of warm physiological saline solution intrauterine, preferably with antibiotics. Treatment may be repeated after a week if no oestrus is exhibited. Favourable results are also reported by

massage of the ovaries. This trait may be an inherited one.

#### Mating at foal heat

Teasing of foaled mare should begin on the third or fourth day after foaling and continued daily until the cessation of oestrus. Usually the foaled mare is covered on the 2nd or 3rd day of oestrus, which usually falls on the 9th or 10th day after foaling. If the mare still remains in oestrus, three days after service, she should be rebred. Two weeks after the last service teasing should be started again. Mares which have foaled recently and which were not served at the foal heat, usually have a normal cycle. However there is a tendency, especially if they are in poor condition, for not to show oestrus again, until a month or more after the foal heat. If a mare foals in poor condition and is on inadequate feed she may enter a phase of true post parturient anoestrus. This is probably the reason, why it has become customary to cover mares during the foal heat rather than to wait for the next oestrus. In thorough bred studs they are keen to see that there is minimum delay after foaling in getting the mare pregnant again and the services are so arranged that the next foaling occurs as soon as possible in the new year.

Service at the foal heat is not desirable. It is often followed by return to service or insecure pregnancy. Mares bred at foal heat often return to heat between 15 to 90 days after service. It is possible that uninvolved uterus may be vulnerable to coital infections. Jennings (1941) in a study of 191 mares, rebred on 9th day after foaling reported a conception rate of 13.7% and an abortion rate of 12.8%, whereas, mares which were bred after longer in-

tervals had a conception rate of 67.3% and abortion rate of 3%. Poor results are due to unsuitable environment in partially involuted uterus and presence of aberrations of ovulation during foal heat.

## Genital Infections causing infertility in mares

### ETIOLOGY AND SYMPTOMS

Genital infections in mares usually occur from two sources. The most common is pneumo-vaginic condition otherwise known as "wind sucking" or aspiration of air into the genital tract. The normal vulva in the mare should be verticle. The vulval lips should appear as firm and well elevated with even closing along the entire length. A flat croup well elevated tail head, sunken anus and small under developed horizontally placed vulval lips predispose to pneumo-vagina. The condition also results due to lacerations, stretching or tearing of vulval sphincter muscles or vulva or perineal portion at the time of foaling. Vulval lips become atropic, flacid and appear to have been rolled in, resulting in gaping. The lips may become sunken below the dorsal commissure and permit soiling of the genital tract with faecal material, air and pathogenic organisms. Once the wind sucking condition develops the habit of aspirating air into the vagina becomes established and in a number of cases it becomes progressively worse. The condition is more commonly seen in old and thin mares; but may also be found in young nulliparous mares. The peculiar sucking and expulsive blowing sounds occur at the time of urination, defecation, at work or when the mare is in oestrus. Wind sucking condition is more commonly observed at the time of oestrus.

Aspiration of air into the vagina may occur in some mares only during oestrous period since at this time the perineal region and the vulvo-vaginal sphincter are more relaxed under the influence of oestrogen.

Wind sucking may occur even in maiden or pregnant mares. Outwardly tipped vulva in maiden mares may act as predisposing factor for pneumo-vagina. Infection of the genital tract may cause pneumo-vagina condition. The condition may be mild, infrequent and not suspected unless the mare fails to conceive. Cultural examination reveals genital infections. Avoidance of a small amount of frothy exudate in the vaginal cavity or of purulent discharge from vulva about five days following coitus is suggestive of this condition. The infection may extend to cervix and uterus. In the wind-sucking mares, the air is sucked through the cervix which may result in ballooning and contamination of the uterus. The pneumo-vagina condition is associated with reflex ballooning of the vagina and the building up of negative pressure in the uterine cavity which normally occur during coitus.

Pathological parturition is a typical source of genital infection in the mare. A dead foal, partially or completely decomposed foetus, retained placenta or severe trauma and incomplete or delayed involution may result in infertility or sterility. In most mares infection of the uterus occurs during the first week after parturition (Bruner, 1954). Infection enters the genital tract when the mare rises soon after foaling and the vagina and uterus balloon with air. In mares in good condition and with healthy uterus this ascending infection is overcome or eliminated by the 9th or 10th day after parturition.

only when there is progressive involution of the uterus and natural resistance to withstand infective condition.

Infections may be introduced into the vaginal passage of the mare at the time of the service due to improper hygienic conditions. Soiled tail, unclean water and appliances may transmit infection to the mare and stallion. Soap or similar detergents and antiseptic solutions used may cause irritation of the vaginal mucous membrane. Dimock and Edwards (1928) reported that 36.5% of 1606 barren mares were found infected. On cultural examination, the following organisms were detected. *Streptococcus genitalium* in 66.4%, *Klebsiella pneumoniae* var *genitalium* in 10.2 % and *Escherichia coli*, *Corynebacterium equi*, *Ps. aeruginosa*, micrococci, *Salmonella abortus-equina*, *Shigella equuli*, chromogenic rods, molds, actinomycetes, staphylococci and streptococci in 23.3%. Dimock and Bruner (1919) examined 2760 barren mares for genital infections and found that 25.7% were infected with streptococci, 2% with *Klebsiella pneumoniae*, 1.9% with *E. coli* and 4% with miscellaneous infections. He is of the opinion that *S. genitalium* is responsible for about 25% of infertility in mares. It is the most common cause of abortion, sterility and disease conditions in foals. The organisms are constantly present as saprophytes on the external genitals of the mare and the stallion gets infected. Normal mares with good resistance overcome this infection within about 10 days following parturition. In mares with poor resistance the infection may remain until the second oestrus and may become chronic under pneumovaginic condition. Infection may not actually prevent conception, but pregnancies are likely to be terminated in abortions or in the birth of diseased

foals. Britton (1947) and Jennings (1941) showed that the incidence of abortions from 25 days to 150 days of pregnancy is greatly increased in mares with genital infection especially of streptococci and when bred during the foal heat.

In the infected mares genital discharge through vulval orifice soils perineal region, buttocks and tail. The vulval lips may gape and expose the mucous membrane. In certain cases vulva becomes loose. The perineal region may look sunken and may become thin and atrophic. The genital tract becomes congested with inflamed mucous membrane. The colour of the cervix changes from pink to brick red or purple. The cervical folds become oedematous and os is relaxed. Mucous membrane becomes moist and blood vessels appear prominent and may even become tortuous. The vagina may show a thin slightly cloudy or a thick yellow white mucopurulent exudate with flakes. In some cases there is a thin and slightly frothy exudate seen coming from the cervix. The above described symptoms, suggestive of vaginitis, cervicitis and endometritis become apparent during oestrus. In chronic cases the exudate is mucopurulent and may be very copious. The cervix is found greatly relaxed, enlarged and becomes indurated. Adhesions may occur in cervix and in rare cases pyometra may result. In chronic cases there is atonic condition of the uterus and the accumulated exudate cannot be expelled. The uterine endometrium may be destroyed over extensive areas and replaced by scar tissue. There is apparent evidence of wind sucking. Diagnosis may be missed in mild or recent cases and as such it is advisable that mares should be subjected to the cervical

mucus cultural examination before they are bred.

*Klebsiella pneumoniae var-genitalium* infection is less commonly encountered than *S. genitalium*. The infection is characterised by a thick viscid, tenacious, slimy exudate containing flocculi. The colour of the exudate may vary from dull grey to yellowish white. Vaginal and cervical mucous membrane appear dull and reddish brown in colour. During oestrus exudate is profuse which may soil vulva and the perineal region. If complicated by the presence of the other infections, inflammation becomes intense involving cervix and uterus, and changes occur in the mucous membrane and the underlying structure. A considerable amount of exudate is found in the uterus. Uterus is enlarged with thickened walls. Occlusion of cervix and development of pyometra are rare. The symptoms and lesions vary depending upon age of the mare, duration of infection, the time of oestrous cycle, the number and frequencies of services during breeding season and the nature of the treatment. The *Klebsiella pneumoniae var-genitalium* is a Gram negative encapsulated rod. It is easily transmitted by coitus, contaminated hands or instruments. Infertility produced in chronic infection tends to last for a longer period or it may even result in permanent sterility. The differential diagnosis of this infection is confirmed by bacteriological examination. The clinical symptoms are not very distinct and cannot be properly differentiated. In suspected mares cultural examination from the cervical mucus should be done as a routine. On a number of occasions mixed infection may be present. Reliable cultural tests can only be done during oestrus. Bruner (1954) has pointed out that

cultures taken from normal mares within the first five days after foaling usually show bacterial growth and are sterile on 7th to 9th day.

### Pathological conditions of genitalia

#### VAGINITIS

This may occur due to various types of infections. However, its incidence is low as compared to cervicitis or metritis. It appears that the vagina is more resistant to infection than the cervix and uterus. It usually occurs as primary infection in pneumovaginic condition and as secondary to severe metritis and cervicitis with catarrhal exudate. Trauma, laceration or severe infection of the vulva and vagina is likely to occur at parturition and may result in chronic vaginitis, pneumovagina and in stenosis. It is likely that the cloudy, turbid, light coloured urine of the mare may be confused with vaginal discharge.

#### CERVICITIS

Variety of infections may cause cervicitis. The mucosa of the external os is usually congested and assumes dark red to purple colour. The os becomes edematous, pendulous and greatly relaxed. In rare instances the cervical glands become cystic; deep suppuration and abscess formation may occur. Laceration during parturition may result in chronic cervicitis, scarring, malformation, incomplete closure of cervical canal or in permanent sterility with development of cervical seal. The cervical mucosa is mainly involved in the inflammatory process. In rare cases adhesions or false membranes may completely block the cervix.

#### METRITIS

Pronounced pathological changes may occur in metritis as a result of various types of infections. Deep red

haemorrhagic areas alternating with areas of whitish yellow coloured mucosa may be scattered irregularly on the endometrium. In severe cases the endometrium presents patchy denudation of mucus membrane with dull granulation tissue. The character of the exudate and degree of inflammation vary from acute to chronic. Cervical occlusion may occur with accumulation of several litres of mucopurulent material in the uterus. In this rare type of pyometra there is very severe erosion and degeneration of endometrium. The uterine wall becomes thin and atonic. In advanced cases the endometrium is practically destroyed; and the mucosa becomes a tough, thick, yellowish white fibrous membrane. In occasional cases of pyometra due to incomplete involution of the uterus with patent cervix the endometrial pathology is not so severe though diffused oedema and patchy areas of sloughing are present on endometrium. Perimetritis in the mare is very rare but when it occurs the results are fatal.

#### SALPINGITIS

Its incidence is rare in the mare. Dimock and Edwards (1928) failed to observe enlargement of the oviducts on rectal palpation in the mare. However, of 83 mares with metritis 21.7% showed evidence of salpingitis as evidenced by gross post mortem and microscopic examination. Salpingitis in the mare is usually of ascending type due to infection from the uterus. In some cases infected oviducts exhibit no gross pathological changes while others showing only catarrhal inflammation. In rare cases inflammation is severe resulting in fibrosis of the oviduct and occlusion of the lumen with inflammatory exudate. The presence of the papillae containing a smooth muscle sphincter through

which the oviduct opens into the lumen of the uterus may be the possible explanation of rarity of salpingitis in the mare. In the mare hydrosalpinx is not recorded.

#### OOPHORITIS

This condition is very rare in the mare. Senile changes may be characterised by atrophy and fibrosis.

#### Prognosis

In mares, vaginal, cervical and uterine infections due to pneumo-vagina or delayed involution of the uterus respond to treatment in early stages. In chronic cases with severe endometrial damage permanent sterility may occur. Massive purulent metritis with cicatrization causing a loss of endometrium and extensive cervical lacerations render the mare permanently sterile. It is difficult and occasionally impossible to overcome infections due to *Ps. aeruginosa*, *E. coli*, *Klebsiella* than *Streptococci* as these organisms are more resistant to antibiotics. In early stages with the mild infection recovery may result within one to two months. In chronic or severe cases, treatment followed by sexual rest for a period of 6 months or more may be necessary. Prognosis is utterly hopeless in pyometra with cervical occlusion.

#### Treatment

Favourable results have been obtained by use of antibiotics and sexual rest. Early diagnosis and treatment of genital infections of mare is necessary to prevent early embryonic death, abortion and sterility.

Most of the genital infections can be prevented by observing strict sex hygiene during breeding operations.



The most favourite treatment during recent years is the use of antibiotics and sulphonilamides for uterine infections, preferably in combination with oestrogens and cortisone. It is experienced in general that broad spectrum antibiotics such as Chlor-tetracycline and Tetracycline are most effective against Streptococci, while as neomycin, streptomycin and aerosporin are most effective against *E. Coli*, *Ps. aeruginosa* and other gram negative organisms. However, broad spectrum antibiotics are also effective against these latter organisms. The specific drug is selected by sensitivity test. The selected drug is infused intra uterine during oestrus and treatment repeated at least 2-3 times. Simultaneous parenteral administration maintains proper blood concentration and promotes rapid recovery. Bacteriological examination of the cervical mucus is necessary after treatment to ascertain that the uterus is free of infection before breeding is undertaken.

#### OTHER INFECTIOUS DISEASES OF GENITAL ORGANS IN MARES

**Coltal vesicular exanthema, genital horse-pox or vesicular vaginitis**

This is a highly viral contagious disease of equines very similar to that in cattle. There are no reports of the spread of infection under natural conditions from cattle to horses or vice versa. However, Hagan and Bruner (1951) cited one reference stating that it was experimentally transmitted from cattle to horses, sheep and goats. The disease is of rare occurrence in United States and is of common occurrence in Southern Germany. It is transmitted through coitus and also by other means. According to Udall (1954) even young foals may be affected. Incubation period is three to six days. There is no fever,

anorexia or signs of systemic disturbances. The disease is characterized by the early appearance of many papules which develop into vesicles of 1 to 3 mm. in diameter and may become confluent. The vesicles rapidly change to yellowish pustules which rupture and produce ulcers. A purulent discharge will appear at the vulva. The vulva is congested, swollen and very painful. There is frequent micturition associated with pain. Tenesmus is occasionally present. Lesions may appear on the skin of the vulva and on healing non-pigmented spots appear and last for several weeks. Healing is usually rapid and may occur even without treatment. In the male similar lesions appear on the prepuce. Breeding activities should be suspended until the infection has subsided and the lesions healed up. There is no evidence to show that the infection causes abortion or infertility. It is necessary to quarantine affected animals for a period of two months. One attack does not confer permanent immunity. Cleaning the affected parts with mild antiseptics and application of antibiotic ointments may be beneficial to guard against secondary invaders. Protection from flies is necessary.

#### Dourine

The disease is due to *T. equiperdum* transmitted by coitus. It is prevalent in Europe, U.S.A. and few other countries. Outbreaks of the disease were reported from Niva California, Colorado, Arizona and New Mexico in 1939-40, affecting mainly the wild horses. Dourine is practically eradicated from the United States.

Only horses are affected by the disease. The infection is transmitted by coitus and by blood sucking flies. The onset of the disease is very slow and the

disease becomes chronic except in the tropics. The incubation period is from one to four weeks or longer. The disease may exist in an animal for months or years and the affected animals alternately show improvement and relapse again. There is progressive emaciation. In the later stages nervous symptoms develop and the condition becomes worse. The early symptoms of the disease are characterised by swelling of the vulval lips and vagina. There is mucus discharge containing trypanosomes. In the stallion the penis prepuce and scrotum become swollen and reddened. The swelling is variable and is free from heat and pain. In the mares genital irritation is evidenced by frequent micturition and switching of the tail. After the acute symptoms subside peculiar raised plaques appear on the skin over the body which are popularly known as Dollar plaques on account of their resemblance to the silver coin. However some may be larger. These appear suddenly and disappear within few hours or days to be replaced again by another crop. The plaques are said to be pathognomic of the disease. Occasionally abortion may occur. There will be depigmentation of the mucosa and skin on the vulva in mare and penis and prepuce in the stallion. There are irregular periods of fever and paralysis of facial muscles, limbs and penis develop gradually. Emaciation is progressive until death occurs. Udall (1954) estimated a mortality rate as ranging between 50 to 70%.

The trypanosomes may be detected in the mucoid genital discharge of the mare and from the urethra of the stallion, in the oedematous fluids in the plaques under the skin and rarely in

blood stream. Diagnosis may be made by complement fixation test.

### Miscellaneous factors causing infertility in Mares

#### NYMPHOMANIA

Cystic condition of the ovaries associated with nymphomania in cattle is not observed in the mares. Rare occurrence of cystic degeneration of the uterine wall probably indicates that cystic follicles similar to those found in the cow and dog can develop.

A peculiar type of nymphomania condition is observed in young middle aged irregularly worked and barren mares which are housed in closed paddocks or stables. The ovaries appear as small, hard and shrunken and not cystic. The affected mares show constant oestrus and refuse to accept the stallion. Conception does not occur in spite of forced service. In this type the mares often become vicious and may bite or kick on slight provocation. Benesch (1955) reported that the only remedial treatment is ovariectomy and treated mares start behaving normally in 3 to 4 weeks time.

#### CYSTIC DEGENERATION OF UTERINE WALL

Proctor (1953) described this condition as rather rare to render the mares sterile. The etiology is not known. The condition is observed in both infected and normal mares. The characteristic symptoms are numerous submucous cysts that vary in size from 1 to 5 cm in diameter and are filled with clear amber coloured fluid. The cysts bulge in the lumen of the uterus. In few cases numerous small, upright, fingerlike

cysts are detectable just beneath the endometrium. The appearance of the endometrium is moist, shiny and glistening. The uterine wall becomes thick, soft and spongy. On rectal palpation the condition may be diagnosed on the characteristic of spongy thick condition of the uterine wall.

### Early abortions in mares

In this condition the aborted or resorbed embryos are not observed. Britton (1947) recorded early abortions between 30 to 90 days of gestation. These abortions were not accompanied by any visible symptoms, except when foetus was expelled. No haemorrhage or uterine discharge was observed. In some cases the foetus got macerated and resorbed. The average annual incidence of this condition varies from 10 to 30% as against 15% in recognisable abortions. Jennings (1941) reported that most abortions in mares occur between 45 and 90 days after conception. In mild outbreaks of equine influenza the annual incidence may rise to 30%. McKenzie (1940) reported an average incidence of 7.5%. He believed that abortions were due to pathologic spermatozoa or ova. Britton (1947) cited some instances of high abortion rates occurring when mares were bred to particular stallions. He also reported abortions in mares due to hypoplastic defective vulva. He recorded poor results when mares were bred at foal heat.

### Tumours of the genital tract

They are rare in the mare. Dimock and Edwards (1928) on their studies of over 2000 mares, reported only six true tumours; of these three were in the ovary and one of which was diagnosed as epithelioma of a firm, lobulated

consistency. The fibrous type of tumours and the granulosa cell tumours occurring in rare instances in young mares can be removed by ovariectomy through vagina. They reported one vaginal tumour as benign fibro sarcoma. Fieldman (1932) reported carcinomas in the ovaries of the mare. Williams (1943) described a dermoid cyst in the ovary and a fibroma of the uterus in a mare. Tumours involving the uterus were leiomyoma, carcinoma and fibroma. Fibroma and carcinoma occurred on the vagina and melanoma, papilloma and sarcoma were commonly found in the vulva.

### Congenital defects

Developmental arrests and other anomalies of the reproductive system of the mare are rare. Hermaphroditism has occasionally been recorded. It is characterised by protruding clitoris and irregular sexual symptoms.

### Persistence of a portion of the hymen

This usually ruptures at the time of first service. Some Veterinarians advise that it should be ruptured with speculum few weeks before service. In rare cases, imperforated hymen in maiden mare requires incision to release the epithelial debris trapped in the vagina and uterus. Tenesmus may be exhibited by these mares with a slight protrusion of distended hymen appearing between the vulval lips.

### Paraovarian cysts

These are remnants of Wolffian ducts located in the broad ligament in the region of the ovary. These cysts are of common occurrence in the mare and they vary in size from 1.25 cm. to 7.5 cm. The cysts are not attached directly to the ovary. Williams (1913) described

large paraovarian cysts in the mare that became attached by a pedicle and caused strangulation of the rectum. Surgical removal of the cyst is the only treatment to prevent strangulation of the gut.

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# Chapter 25

## Infertility in Sheep and Goats

The problem of infertility in sheep and goats is comparatively of a much less magnitude than in cattle and buffaloes. Although the anatomical structures and physiological functions are more or less the same as in the cow, the smaller size of ewes and does renders difficulties or impossibilities in adoption of certain procedures and types of therapy. Though sheep are considered as relatively fertile animals, annual losses from infertility to the extent of 5 to 10% are reported from British Sheep flocks. On account of the low value of individual sheep and the bare fact that the infertile ewes are only recognised at the end of lambing season, veterinary advice is rarely sought from those which do not settle to

fied as hereditary or congenital and acquired type due to environmental, nutritional and infectious factors.

### Anatomical or structural defects

These are rare in female sheep and goat except for fair incidence of intersexes or pseudohermaphrodites in goats. Hermaphroditism was noted most commonly in Saanen and other polled goats (Eaton, 1913 and Asdell, 1941). In one Saanen herd, an incidence as high as 11% was recorded by Eaton (1913). The intersexes are usually male hermaphrodites. The condition appears to be inherited as a simple recessive character associated with hornlessness. Horned hermaphrodites are rare Eaton (1913)

in the development such as uterus unicornis, uterus didelphys, persistence of the medial wall of the Mullerian duct, the fleshy pillar caudal to the cervix and persistence of hymen. Bhole (1967) recorded defective development of tubular genitalia, uterus unicornis and two cases of segmental aplasia out of 280 sheep genitalia from abattoir. Occlusion of the oviducts and hydrosalpinx is an occasional cause of infertility in ovines.

### Functional defects

Functional ovarian activity rarely causes breeding difficulties. Ewes usually experience one or two oestrous cycles at the beginning of the breeding season at which they do not exhibit oestrus but start settling down to normal cyclical activity. It is however evident that ovarian activity is usually suppressed by submaintenance diet or deficiency of various types.

Anestrus condition is not so common in sheep except in unthrifty ewes which are usually culled. It is natural that when rams are turned out with the flock it is usual for most of the ewes to be bred within a month or so. In some ewes the first few heats of the breeding season are not accompanied by ovulation and many ewes frequently fail to conceive to early heats than at later matings. Exact cause of the first few unovulatory heats is not known (Dutt, 1954). There is possibility that a greater degree of embryonic death may follow early matings.

From the experiments conducted by Hammond (1921), Winters and Feuffel (1936) and Arthur (1956) it is evident that embryonic deaths or resorption of foetuses is a conspicuous feature of sheep infertility. By comparing the number

of corpora lutea with the number of foetuses they estimated an incidence of 8 to 13% of this condition. Such pathological reproduction is usually encountered more with multiple than with single conception. Sporadic cases of foetal mummification are occasionally seen. Cystic condition of the ovary is rarely recorded and is not a major cause of infertility.

A specific environmental cause of sheep infertility is reported from Australia. This was due to grazing on pastures of subterranean clover which contains large amounts of estrogenic substance (genistein 100 mg per 100 gm of clover), ingestion of which leads to cystic degeneration of the endometrium resulting in permanent sterility. Bennetts *et al* (1946), Newson (1953), Schinckel (1948), Underwood and Shier (1951) reported an incidence of sterility in ewes due to feeding of subterranean clover to the extent of 30%. Dystokia occurred due to uterine inertia accounted for 30 to 40% of the foetuses being expelled or removed dead and for the death of 15-20% of the ewes. Prolapse of vagina occurred in 10-12% ewes. Schinckel (1948) and Underwood and Shier (1951) reported that infertility in the ewes fed on subterranean clover persisted even when the affected were transferred to pastures free from subterranean clover. Oestrus and ovulation occurred regularly but fertilization or implantation did not occur due to the pathologic 'Swiss Cheese Type' endometrium. Curnow and Bennetts (1952) and East (1954) reported that the toxic principle in the Dwalgan up strain of subterranean clover was an isoflavone derivative [(5,7-*H*) Trihydroxy isoflavone]. That was one fifth as potent as estrone in estrogenic activity.

### Nutritional deficiency in relation to infertility in sheep

Nutritional deficiencies in sheep more or less produce similar effect on reproduction and fertility as in cattle. Deficiency diseases affecting reproduction in ewes on range involve lack of minerals especially phosphorus, cobalt, protein and Vitamin-A. The inanition effects on fertility are more pronounced in immature than in adult sheep. The effects are characterised by failure of estrum. Voluminous data on the practice of flushing sheep indicated that flushing or the provision of a greatly increased supply of good quality roughages or grains, preferably the latter results in an increased intake of the total digestible nutrients which promotes fertility rate and a greater lamb crop, e.g. gain in weight, is also significantly greater than in the unflushed ewes. It is considered that a flushing period of about two weeks or so is sufficient before the rams are introduced into the flock. In case ewes are in a state of high nutrition, flushing has very little effect on the lamb crop which neither increases nor decreases the fertility. (Reid 1949 and Miller *et al*, 1942).

### Effect of plane of nutrition during pregnancy

Miller *et al* (1942) were able to increase the lamb crop in ewes reared on range condition from 80 to 135% for a period over six years, by continuously maintaining the ewes on high plane of nutrition.

Stamp (1963) stated that feeding ewes in early pregnancy is relatively not so important but failure to maintain desirable weight in ewes during late pregnancy can lead to considerable foetal and ewe mortality.

There is not much evidence to prove that high plane of nutrition causes infertility or sterility. Reid (1949) pointed out that symptoms of Vitamin A deficiency in the sheep are similar to that of cattle. Ewes deficient in Vitamin A usually abort or deliver dead or weak young ones. In sheep the female is more susceptible to Vitamin A deficiency than male. In drought years feed is usually low in Vitamin A, carbohydrates, protein and phosphorus. Severe cobalt deficiency may lead to debility, inanition, failure of oestrus and birth of weak lamb crop.

Bowstead *et al* (1942) reported that postnatal loss of lambs from ewes fed on low level of cobalt was 58% as compared to 17% from the lambs nursing the ewes which were receiving cobalt supplement in the field.

Reid (1949) pointed out that reproduction in sheep is not affected until the usual symptoms of phosphorus deficiency develop to a proper degree. In sheep, symptoms of phosphorus deficiency are similar as in cattle, Miller *et al* (1942) showed that a low protein diet was not so severe as one low in both protein and phosphorus. Reproductive symptoms of phosphorus deficiency are similar as in cattle. Miller low protein and Vitamin A level are delay in the onset of puberty and estrus, irregular estrous cycles and a tendency to produce only one lamb on alternate years. Abortions are not common but weak or dead is usually due to a lack of the minerals in hay or pastures. Fertility will not be impaired when the ewes or does are receiving protein supplement and mineral containing phosphorus. In cases of severe mineral or protein deficiencies ewes may pull out and eat the wool of other sheep in the flock.

The growth rate of foetal lambs increases rapidly towards the end of gestation period and its energy requirements are probably met largely from glucose. Part of glucose is converted to lactose and this cannot be returned to maternal circulation despite severe maternal hypoglycemia. Near full term, the foetal needs for glucose may be as high as 40 grams/day and the maternal glucose is mainly derived from propionic acid which is only a minor fraction of the volatile fatty acids produced by ruminal fermentation. It is therefore necessary that ewes should be fed on increasing amounts during the pregnancy to provide for the foetal requirements of both glucose and amino acids. Ewes carrying twins have to meet still larger demands.

Under-nutrition during late pregnancy may lead to hypoglycaemia, hyperketonemia, reduced birth weight of lambs and delayed lactation which is usually accompanied by the impaired condition of the ewes. The occurrence of pregnancy toxemia due to under nutrition indicates that the remainder in the flock is also being affected.

### Copper deficiency

Bennetts and Beck (1942) and Moule *et al* (1959) reported that a low copper status of pasture and of pregnant ewes associated with either inadequate or adequate copper level in the soil had been responsible for neonatal mortality amongst lambs in Western Australia and in Queensland.

### Iodine deficiency

There are a number of reports of goitre in new born lambs fed on Kale and Brassica crops indicating a very high lamb mortality towards the end of pregnancy, due to iodine deficiency.

### Effect of light

It was observed by Bissonette (1936, 1941) that sheep, and goats breed during the period when there is decrease in day light. He bred Toggenburg goats in July after placing them on a reduced light schedule from April to July. The control group of goats showed anestrus condition from March to September. Eaton and Simons (1953) made similar observations. The birth weight of lambs and kids born in the fall was lower than the birth weight of the ones born during spring. The mortality at birth and in the new born was greater in the fall lambs and kids. Bissonette (1936) showed that the effect of the light on inducing estrus and estrous cycle was mediated through the pituitary gland. By reducing intensity and exposure to light pituitary is stimulated for release of gonadotropic hormones which in turn produces normal estrus. The increase in light apparently causes an increased secretion of FSH. The reduction in light may reduce the amount of FSH secreted but, it more likely increases the amount of LH and thereby initiate onset of breeding season and ovulation (Kammlade *et al* 1952).

### Ambient temperature

There is evidence that low reproduction rates of flocks in pastoral Australia are associated with high ambient air temperature (Maule, 1958). His observations indicate that the high air temperature experienced in semiarid Queensland can lead to neonatal mortality.

Roy (1969) reported that the comparative fertility of non lactating, Bikaneri, Mandi and cross breeds (Corriedalex Bikaneri) when bred in summer was equally high. Thus the results of studies in U.S.A. and Europe in which



early stages of pregnancy have been found to be particularly sensitive to high temperature do not apply to the same degree to the indigenous sheep with their origin in hot areas. He obtained high lambing percentages in the indigenous sheep even when the ewes were bred during the hottest part of the year with successful continuation of pregnancy during subsequent months (Autumn) which were also hot, provides justification that fertility in indigenous sheep does not decline in summer months than in ewes bred at naturally occurring estrus. Hammond *et al* (1942) observed that pregnant mare serum and horse pituitary extracts induced estrum only in ewes having regressing corpus luteum. Ewes in which there was absence of corpus luteum, ovulation occurred without estrum. Robinson (1950), (1954) made similar observations. In the presence of an active corpus luteum however, both estrus and ovulation were usually suppressed. Murphree *et al* (1944) reported that ovulation could be induced experimentally in both follicular and luteal stages of the oestrous cycle of the ewes but the ova liberated during the luteal phase had a very low incidence of fertilization. Venzke (1953) reported that a group of 77 ewes which was treated with estradiol (E.C.P.) dose varying from 2 to 7 mg., during the anestrus period 90.9% came into oestrus but only one ewe settled to service and lambed.

Cystic ovaries are rare in ewes and goats but it may occur in goats with high milk production.

#### Prenatal or Embryonic death rates in Ewes

Hammond (1921) observed in 80 pregnant ewes that there were normal

foetuses equal to only 87% of the corpora lutea present. The number of atrophic foetuses and missing eggs were about equal in number. Henning (1939) reported on the incidence of foetal deaths that 16% of the corpora lutea could not be accounted for, by live foetuses. He noted that early embryonic death with complete resorption could not be detected. He drew attention to the increased foetal deaths with the increase in number of ova liberated: 8% with one ovum, 26% with two and 43% with three ova. Casida (1953) reported on his work that overall embryonic death rate on the basis of 18 day non return was 20% but that on the basis of lambing rate the embryonic death rate reached 30%. Morely (1954) reported a prenatal death loss in Merino sheep in Australia of about 25 to 30% extending from three weeks after conception to term. Dutt (1954) reported on his observation on 180 North-Western ewes that when they were bred once early in the breeding season (August and September), showed an embryonic death rate of 20% and failure of fertilization in 38.9% of the ewes. Failure of ovulation was uncommon in ewes bred early in the breeding season, but failure of fertilization was most important factor accounting for the low lambing rate.

#### Early Embryonic Death in Does

Achuthan Kutty and Raja (1971) studied 175 gravid uteri with oviducts from Trichur abattoir. Early embryonic mortality was detected in 23.29% of the genitalia studied. Out of the overall prenatal loss of 14.9%, 11.1% occurred before 40th day of gestation. The estimated actual loss of embryo after 40 days of gestation was 5.6%.

### Pathological conditions

Nair and Raja (1972) studied pathological conditions of the Caprine female genitalia by studying 1860 specimens including 400 gravid ones from Trichur abattoir. The overall incidence of lesions was 2.15%. The following conditions were recorded:

Cystic ovary	3.032%
Ovarian abscesses	0.053%
Hydrosalpinx	0.107%
Cysts in the salpinx	0.053%
Salpingitis	0.053%
Perisalpingitis	0.053%
Macerated foetus	0.752%
Pyometra	0.322%
Hydrometra	0.161%
Metritis	0.053%
Perimetritis	0.053%
Parametritis	0.053%
Haematinic mummification	0.053%
Interplacental haemorrhage	0.053%
Uterine rupture	0.053%
Retained foetal cotyledons as	
	0.106%

Nair and Raja (1973) studied 338 gravid genitalia from goats ranging from 15 days to term from Trichur abattoir. The embryonal loss was evident in 18.48%. The loss during the first 40 days of gestation was 10.79% as against the estimated loss of 3.85% beyond 40 days of gestation. The ovum embryo loss was found to be much higher 33.33% in multiple ovulation when more than 2 ova were shed.

### Effect of flock size

Stamp (1963) stated that lambing percentage in given breeds tend to vary inversely with flock size.

### Influence of Age

Stamp (1963) stated that on an average, the percentage of lambs born increased to 4th lambing.

### Seasonal influence

Numerous studies show that efficient breeding at highest fertility level in both male and female occurs at definite periods of the year. A number of adverse factors operate both prior and subsequent to this period namely silent heat, non fertilization of ova, failure of ovulation, low ovulation rate and early embryonic death.

### Genetic Factors

Stamp (1963) stated that prolificacy is a character of heritability. Experience suggest that the actual heritability of prolificacy in the majority of single lambing is of the order of 5% when differences of prolificacy among the sheep of same breed are under consideration. The figure is probably high in some cross breeds since there is a considerable breed to breed difference in prolificacy. In few breeds the actual heritability of prolificacy may be considerably higher than five per cent as in Cheviots. There is very little evidence of an inverse correlation within breeds and in crosses between the extent to which a ewe face is covered with wool and its reproductive performance. There is no evidence to suggest that infertility is the consequence of breeding for some other characters such as wool and milk. In few breeds, selection for given characteristics namely large head and shoulders is the cause of high incidence of dystokia.

### Endocrine abortion in Goats

Vanheerden (1963) reported a condition as endocrine abortion in Angora goats. It is characterised by marked regression of the corpus luteum and exhaustion of acidophil cells of the anterior pituitary which secretes-Luteotropic hormones (LTH). At the same time there is secretory activity in the

pituitary basophil cells which produce FSH and LH hormones and this is associated with ovarian follicular development and estrus at the time of abortion. The condition causes abortion and is believed to be due to hereditary effect of the anterior pituitary to maintain corpus luteum of pregnancy.

### Infective factors causing reproductive disorders

- (1) Enzootic abortion of ewes (E.A.E.) virus.
- (2) *Vibriofetus*.
- (3) *Toxoplasma*.
- (4) *Brucella melitensis*.
- (5) *Brucella ovis*.
- (6) *Salmonella abortus ovis*.
- (7) Tick borne fever virus.
- (8) *Listeria monocytogenes*.
- (9) *Clostridium chauvoei*.

} Un-  
common

Most of the infectious diseases associated with reproduction either cause abortion or premature lambing, and as such differential diagnosis is often difficult.

### Enzootic virus abortion of ewes (E.A.E.)

#### EPIDEMIOLOGY

The causative agent is a virus of the Psittacosis lymphogranuloma group. The disease is reported from most of the European countries, Canada, New Zealand, South Africa and U.S.A. The viral infection is introduced in clear flocks by the purchase of infected sheep which may be of any age. The dissemination of the virus within a flock takes place only at the lambing period and during the few weeks before this when abortions are occurring. The introduction of infected sheep in a clean

flock does not lead to clinical disease within the home flock until the second lambing occurs. The infection is not spread by the ram. The virus is disseminated at lambing time not only to mature sheep but also to yearlings and new born lambs and in turn these also abort at their first lambing having carried virus in the tissues for one or several years. The abortion rate varies from 5 to 25% and continues year after year in infected flocks. Many more sheep are usually infected than the number which actually aborts.

### Clinical symptoms

The only clinical sign of the infection is abortion or premature lambing which occurs late in pregnancy usually during the last two or three weeks. Majority of the foetuses are born dead but are fresh. Only a few obviously appear to have been dead in the womb for some time and show signs of putrefaction. In addition to the abortion a number of weak lambs are born alive either at full term or only a few days premature. After delivery there is a varying amount of pink creamy, vaginal discharge and the placenta may be found retained. Deaths in aborting ewes are very few and later, the aborted ewes do not necessarily become sterile. Sheep of all ages are liable to be affected but the heaviest infection occurs in ewes carrying their second lamb.

### Diagnosis

Diagnosis may be arrived at from

- (a) Characteristic appearance of placenta.
- (b) Demonstration of viral elementary bodies in Zeihl Neilsen's stained smears of placenta pre-

ferably examined under dark field illumination

### (c) Complement fixation test

### Control

Vaccination of ewes using adjuvant vaccine containing large amounts of killed virus gives necessary protection

### *Vibrio foetus*

#### EPIDEMIOLOGY

*Vibrio foetus* infection in sheep appears to be prevalent in many countries of the world. The incidence of abortion in affected flocks varies considerably. Stamp (1963) considered that the losses due to *vibrio foetus* infection are rarely heavy, the incidence of abortion being near about 5 to 10%. However, out breaks with a higher incidence of 50% or in some as high as 80% to 90% have been reported by Blackmore and Gledhill (1946). In the same flock the disease is rarely seen two years or more in succession and usually it does not lead to any uterine complications resulting in infertility. Ewes aborting from vibriosis become immune at least for one year. There is evidence that standing surface water or contaminated shallow well may be reservoirs of infection. It is therefore necessary that pregnant ewes should only have an access to clean running water (Hadleigh 1956). It is difficult to infect a ewe at breeding time by any method of inoculation. The susceptibility of the ewe appears to be relatively low until she has been pregnant more than a month.

Ovine vibriosis is definitely not a venereal disease and is not transmitted by the ram for it would seem that wide spread infection causing abortion throughout the flock can occur during late pregnancy. Infection probably

occurs through digestive tract. Very little is known of the existence of carrier animal or other natural reservoirs of infection. The fact that out breaks of abortion are confined to one lambing season and the failure of the disease to recur during the second lambing season might suggest the possibility of an absence of continuing infection but evidence is suggestive that it is more likely due to solid immunity.

#### Pathogenesis

When animal is infected with *V. foetus* it involves uterus particularly at the cotyledonary sites. According to Smith (1919) infection is gradual and the number of the affected cotyledons increase progressively. In the affected areas the foetal blood supply is destroyed and abortions ensue. In ewes abortions may occur at any time during pregnancy but usually during the last month. Even though a very large number of organisms are often found in foetal tissues they do not appear to cause disease of the foetus itself. The pathogenic effect of the organism is extended on the placenta. No definite information is available concerning pathogenesis by *V. foetus* in the non gravid uterus. When vibriotic infection interferes with fertility otherwise than by causing abortion it possibly does so by producing intra uterine condition which prevents conception or implantation. There is no evidence that *V. foetus* invades any tissue other than genital tissue in the female. No pathogenic effects appear in the ram. Evidence suggests that when infection is present male animals act as passive carriers (Sjollema *et al.*, 1949; Buxton 1950). The site of infection in the male is not definitely known but *V. foetus* has been isolated from the heart blood

and liver of ram. Vaginal mucus agglutination test is often useful in the female.

### Clinical Signs

The usual history given by the farmer is that a few lambs have aborted in early stages of pregnancy but the abortion storm usually begins at about six weeks before the normal lambing time. Abortion usually occurs one to three days following death of the foetus so that the foetus is always born dead with signs of decomposition. Weak lambs is not a characteristic of this infection.

### DIAGNOSIS

Diagnosis may be arrived at from :—

- (a) Demonstration of *Vibrio* in placental smears or in the smears from the stomach contents of the foetus.
- (b) Culture of the organisms from foetal stomach contents.
- (c) Serological tests are of doubtful value.

with abortion or neo-natal mortality of lambs. Abortions usually commence 30-40 days before parturition and continue until full term. The aborted foetus may be found degenerated. Lambs at full term may be born alive or dead. Some lambs, though born alive may succumb within 3 to 4 days of birth. In twin lambs it is quite possible to find one normal and the other one affected. Prenatal mortality in affected flocks may be as high as 50%.

### Diagnosis

Diagnosis is done from the foetal cotyledons by the presence of—

- (a) Many small white necrotic foci.
- (b) Mouse inoculation followed by the demonstration of pseudo cysts in the brain.
- (c) Antibodies by the Sabin Feldman dye test of ewe's blood.

### Control

### Diagnosis

Diagnosis is usually arrived at by:

- (1) Circumstantial field evidence including high fever in the sheep.
- (2) There is absence of pathogens in foetus or placenta.
- (3) Demonstration of tick borne fever bodies in blood leucocytes.

### Control

Antitick measures should be undertaken.

### Brucella Infection

In sheep, abortions may be caused by several members of the *Brucella* group namely *B. Ovis*, *B. Melitensis* and *B. Abortus*. In all the three types the clinical symptoms are similar. The organisms cause death of the foetus late during pregnancy and considerable prenatal mortality occurs at lambing time. *B. Ovis* also causes epididymitis in the rams. *B. Melitensis* was detected in goats by Zammit (1905). This was the beginning of the knowledge that undulant fever in man was caused by *B. Melitensis* and the spread was chiefly through infected goats milk. The disease is endemic in goats in many of the mediterranean countries and is also found there in sheep. In goats the symptoms vary. Although abortions are of recurrent nature it is not a constant feature. Symptoms of generalised malaise with pyrexia are observed. Milk of affected animal may change to a clear fluid containing clots. Abortion and parturition subsequently lead to persistent vaginal discharge.

### Diagnosis

Diagnosis can be done by:

- (a) Demonstration of the organisms

in placenta either by smear or by culture.

- (b) Serological diagnosis using the agglutination or complement fixation test.

Investigations done under the infectious abortions scheme in Kerala indicates the presence of *Brucellosis*, *Vibriosis* and *Leptospirosis*.

171 Caprine sera were collected for examination of which 93 were from goats belonging to the goat project for milk production and 78 from slaughter house Trichur. Of these 12 were suspected and 11 of them had low titre and one had high titre for *Brucella*.

Kidney of one goat had suggestive lesions of *Leptospirosis* and a strain of *Leptospira* was isolated at Trichur. WHO reported that this might be a new bio type of *Leptospira* which has not been isolated till now from any country.

### Control

Vaccination of rams by strain 19 vaccine along with adjuvant vaccine of killed *B. Ovis* gives better results. It is important to control the disease in the male so that infection will not occur. Vaccination of female is of very limited value.

### *Salmonella abortus ovis*

In the infected ewes abortion occurs late in the pregnancy and there is considerable prenatal lamb mortality.

### Diagnosis

Diagnosis may be arrived at by—

Culture of the casual organisms from the placenta and of foetal stomachs.

## Control

Vaccination with formalised whole culture has been used and appears to be of some value. However it does not always give satisfactory results.

## Ulcerative vulvitis

This is described as ulcerative dermatosis (Tunncliffe, 1949), and as necrotic venereal disease (Newsom, 1953). The spread is by an infected to a susceptible ewe causing a necrotic swollen bleeding lesion on the skin and mucous surfaces of the vulvar lips. The lesion usually starts at the lower commissure of the vulva and spreads upwards. Occasionally it may spread to vagina. It spreads to the ewes at the time of coitus by an infected ram that has lesions on its penis and sheath. The disease may spread to wethers and to ewes that have not been bred. The symptoms usually subside within a week's time. 5% copper sulphate solution has been advised for treating these lesions. It should be only applied externally and should not be injected into vagina.

Williams (1913) and Hutya *et al* (1946) reported that vesicular venereal disease or coital vesicular exanthema may be occasionally spread through flocks of sheep and goats with similar symptoms as in cattle and horses.

The above two diseases although may cause cessation of breeding until lesions are healed, are not characterised by infertility. In acute stage ewes may refuse to copulate due to irritation and pain.

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# Chapter 26

## Infertility in the Sow

As compared to the other farm animals information concerning infertility in the swine is rather scanty. The prolific rate of reproduction permits intensive culling of sub-standard and infertile ones, in consequence of which extensive studies on the problems of infertility have become difficult.

On account of the diagnostic difficulties in the living ones information with regard to infertility in the swine have to be obtained from the abattoir material. Drastic culling of unproductive ones prevents systematic studies of various factors concerning infertility during various stages of development in living animals.

Increasing demands for meat during last few decades has led to commercialisation of swine breeding. In this system multiplication of this species in an entirely artificial environment and within the programmed frame work together with economic consideration is usually practised. In this changed situation the research activities are mainly directed towards reproduction and not much on infertility or sterility

1.5% of the 5088 sows and gilts. In a detailed study Wilson *et al* (1919) and Nalbandov (1952) reported that 21.5% of the infertility cases were of congenital origin due to hydrosalpinx. They attributed the cause to the obstruction of oviduct consequent to closure of Wolffian duct during embryonal development.

Becze and Szilvassy (1976) recorded the incidence of tubal abnormalities in anoestrous animals to the extent of 1 to 2%.

True and pseudo hermaphroditism are reported in swine, the incidence is hardly 1%. Segmental aplasia of Mullerian ducts, cervix duplex and unilateral or bilateral hypoplasia of ovaries is also recorded (Figs. 86, 87).

All reproductive organs missing except vulva	0.04%
Double cervix	0.04%
Double uterine horn	0.02%

There were 1.8% open gilts, 0.6% pregnant gilts and 1.2% of the open (breedable) sows or a total 1.5% of the 5088 sows and gilts with tubal abnormalities. Animals having certain types of congenital abnormalities do breed but the low litter size is an indication of the existence of such malformations. Physically well developed sows are usually selected for breeding irrespective of their reproductive abilities. As such those having inherent defects are responsible for perpetuating the sublethal characters in progeny.

### Variations of the Sexual cycle

Variations of the sexual cycle should be judged in the light of the observations concerning seasonality of oestrus and oestrous cycles. Swine is a poly-oestrus animal having 21 day long cycle and apart from certain seasonal differences it is also fertile all the year round. This is evident by the fact that farrowings occur in any season of the year with slightly variable litter size. However, inconsistency in the duration of oestrus and intensity of the symptoms during oestrus have been observed. A peculiar trend is observed in swine that shorter or very intense or very mild heats may follow each other without any regularity in the same individual. Durations of oestrous cycle also vary in the same individual. One or more silent heats can be observed in sows having long anoestrous condition. The incidence of irregularly long or short cycles is higher in sows than in other species.

Observations on large number of successive cycles followed for years have

shown a well expressed tendency according to which duration of heats and the intensity of their symptoms decrease proportionally with every farrowing and the cycles become longer. Weakening of sexual functions with each farrowing has also been observed by du Meznil du Buisson and Signoret (1968) in a small number. However in other sows, anoestrus may occur after 2-3 heats following farrowing.

There is a seasonality in gonadotrophic function in the swine. The number and fertile heats in winter and early spring months are higher than in other months of the year (Cole and Cupps, 1959; Bishop, 1963; Aamdal, 1967 and Roberts, 1971). The ovulatory response in sows to gonadotrophic treatment is maximum during March and April (35.3 to 38.5%) and minimum during May and September (10.4 to 14.7%) (Webel *et al*, 1970).

Anovulatory oestrus occurs in fusarium toxicosis and cystic degeneration. Even in sows exhibiting oestrus, the heats may be anovulatory. The incidence of anovulatory heats in sows is low as compared to other species.

In some sows which have not exhibited heat previously, quiet ovulations were seen on post-mortem examination. Silent heats follow in those sows which have expressed 2-3 successive normal heats after farrowing. Following MAP treatment, if PMS is administered on the day of withdrawal of MAP and not on the following day, oestrus will not be observed in 20 to 25% of sows but ovulations do occur resulting in pregnancy (du Meznil du Buisson, 1970). In sows the time of ovulation is related to onset of oestrus and it is independent of the duration of oestrus. This is in agreement with the finding that dura-

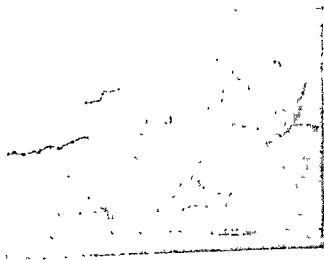


Fig. 84 Tuberculous endometritis in a buffalo.

← Fig. 85. Cystic ovarian degeneration in the right ovary of a buffalo.

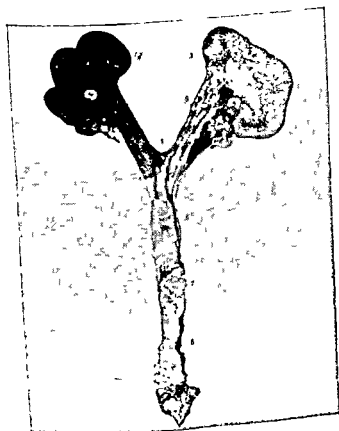


Fig. 87. Unilateral pregnancy (left) with aplasia of right uterine cornua in a sow.

← Fig. 86. Segmental aplasia of uterine cornua in a sow.



Fig 87 Ovary of the sow 45 hours after onset of heat showing ovulated follicles corpora haemorrhagica and follicles that failed to ovulate



Fig 89 Ovary of the sow 79 hours after onset of heat showing ovulated follicles corpora haemorrhagica and follicles that failed to ovulate



Fig 90 Multiple large cysts with a single corpus luteum in the sow



Fig 91 Two large cysts with a single corpus luteum in the sow

tion of oestrus does not influence the length of cycle (Becze *et al*, 1975).

Ovulatory potential of the ovaries is genetically determined like ovarian rhythm. Even among paternal halfsibs, significant differences can be demonstrated in respect of the number of ovulations (Smidt, 1969). The actual number of ova shed is however controlled by several endogenous and exogenous factors. In gilts the number of ovulations is low-12 to 15 and this number increases upto sixth farrowing and then decreases with advancing age of sows.

Number of ovulations is rather low in cycles following early weaning. Flushing as well as caloric improvement in diet increases the number of ovulations. However it does not increase the litter size since this causes more embryonic losses (Zimmerman *et al*, 1963 and Rigor *et al*, 1963). Some of the follicles even on attaining full size may not ovulate. Some of the follicles may not show ovulatory point and will persist for longer time without ovulation. These can be observed upto 40th to 50th day of pregnancy. Their number on an average is two but may be even higher viz. 4 to 6 (Becze, 1968 and Nguyen Thein, 1974) (Figs. 88, 89).

Not all of the bled-in and luteinised follicles ovulate. In order to confirm the difference between number of corpora lutea and the number of ova shed, Paraipan (1971) observed no ovulatory stigma on the follicles in spite of the presence of preovulatory in-bleedings. Cumulus oophorus showed characteristic disintegration and in several cases the ova was freely suspended in the liquor folliculi. Intra follicular luteinisation occurred relatively early in these follicles. These become atretic and are difficult to be differentiated from a

normal C.L. However they contain a degenerated ovum. This phenomenon occurs in 16% of follicles. Besides normal ovulation, cystic degeneration of some follicles may occur without interfering with normal oestrous cycle.

### Ovarian cysts

In swine one of the factors concerning infertility is the cystic degeneration of the follicles. Generally cysts develop from the un-ovulated follicles. These cysts are classified according to the histological picture. These include (i) follicular theca cysts with more or less intact granulosa (ii) theca luteira cysts in which the follicular epithelium is more or less luteinised and (iii) older cysts in which the functional elements of the follicle are missing. The wall of the cyst consists of only the basal membrane and fibrous tissue. Cystic corpora lutea have never been recorded in swine. The following conditions are responsible for the formation of cysts (i) disturbed relation between pituitary and hypothalamus, (ii) imbalance of FSH and LH ratio or (iii) LH insufficiency. The role of thyroid and adrenal cortex is not very clear in this context. The disturbance in pituitary and hypothalamus relationship appears to be more obvious because cysts can be induced by administration of P.M.S. in pregnant as well as non-pregnant sows (Roberts, 1971). Cystic degeneration of the follicles is also frequent in case of hypophysis blockade produced by progesterone or synthetic gestagen treatment (Neller *et al*, 1961; First *et al*, 1963; Kilpatrick *et al*, 1963). It has been demonstrated that above mentioned both hormonal treatments disturb the endogenous FSH/LH ratio. According to Roberts (1971) small and large multiple cysts in sows may be due to increased

thyrotropic activity. Nalbandov (1964) has described the classification of the ovarian cysts as under.

### Retentional cysts

Some of the follicles do not ovulate but grow further during the early luteal phase and later they undergo regression. Such cysts are 2-3 cm. in diameter and occasionally occur in pairs. They do not influence sexual cycles and hence these are not considered as abnormal.

### Multiple giant cysts

These are much bigger than the follicles which ovulate and even they can reach the size of 10 cm. The affected ovary will become very big and will appear like a cluster of grapes (Fig. 90). The ovarian tissue will be reduced because of pressure atrophy. The reason for their origin may be attributed to insufficiency of L. H. Granulosa layer in the wall of the cyst is completely or partially luteinised. Granulosa tissue can be found in some areas only, usually with the signs of degeneration and desquamation. Luteinisation is more common in medium and smaller cysts. Such cysts appear like yellow bodies to the naked eye. Granulosa and theca layers also take part in luteinisation. In other cases luteinisation will be found to occur in isolated areas. The walls of the large cysts consist of only fibrous tissue as the granulosa and theca interna layers degenerate because of the increased distension of the cysts.

The oestrogen production by the large cysts is poor whereas luteinised cysts produce progesterone and maintain secretory status in the uterus. In the case of large cysts irregular cycles with usually prolonged periods of

anoestrus is observed. It is not clearly known whether the apparently normal yellow bodies that are present in some cases in addition to large cysts are from the previous cycle or from the ovulations from abnormal cycles. It is also not clearly known whether these occur as a result of complete luteinisation of the smaller cysts.

### Multiple small cysts

These are less frequently observed. They are hardly larger than the mature follicles (Fig. 91). Their number exceeding the number of ovulations is usually observed since these can be produced experimentally by P.M.S. treatment; probably they develop as a result of temporary or permanent over production of gonadotrophin. Both the naturally occurring and experimentally induced small cysts are similar to the intact follicles. They contain and excrete more estrogen than follicles. Animals with such cysts exhibit very irregular duration of estrus and oestrous cycles. Even in spite of multiple small cysts, no nymphomania is observed. However, estrogenic effect is seen in the endometrium.

Nalbandov (1952) has recorded that occurrence of cysts is more common during spring and the chances of recovery are very remote.

Classen (1968) studied the histology of the cysts and the endometrium and found luteinized follicular cysts in 45%, follicular theca cysts in 38%, intermediate forms between follicular theca and luteinized follicular cysts in 9.7% and other cysts in 12.9% of the total cases of cysts.

In the luteinized cysts, the lutein tissue often shows the signs of degeneration and it is oedemic towards the

centre. The theca externa is often infiltrated with leucocytes and lymphocytes and fibroblasts infiltrate from the stroma. Degenerative changes occurring in the follicle may probably be the result of compression atrophy.

The follicular theca cysts in some cases have intact granulosa cells which hardly differ from the follicle, whereas in other cases it is degenerated to a varying degree. Instead of 10 to 12 layers which is usually found, it will be 1 to 2 cell layer in such cases and in some other cases it separates and remains isolated. Granulosa cells undergo vacuole formation, plasmolysis, karyorrhexis and pyknosis. The condition of theca interna depends on that of granulosa, the more advanced the degeneration of the granulosa cells the more the theca interna cells lose their epithelial character and become fibrosed. The theca interna proliferates into the walls of the cysts along with the capillaries. The theca externa is oedematous, there is cystic degeneration in the tertiary follicles found in the stroma. The histological picture of intermediate form of cysts reveals early luteinization. The granulosa cells become larger, their granulosity disappears and either luteinization occurs or vacuoles develop. At some places where luteinization is advanced, capillaries will then penetrate through the basal membrane. Mitosis in the tissues which is a normal phenomenon during the development of corpus luteum is never seen but all forms of cell degeneration are present. In some cases, the granulosa character remains dominant thus the luteinization of granulosa does not take place but atypical luteal tissue will be formed by the penetrated theca elements. Besides this, the theca externa is intact or is infiltrated with stroma cells.

In the dead cysts of unknown origin follicular epithelium is missing. The wall of these cysts contains fibrous tissue which is poor in cells and often oedematous. In the wall of the cysts dilated capillaries containing detritus are found.

The overall study of endometrium in respect of various cysts has revealed the following incidence of the cycle; follicular phase 2%, ovulatory phase 14%, luteal phase-I, 50% and luteal phase-II, 10%, involutory phase 17%, and 7% could not be evaluated. In 14% of the cases, cystic endometritis occurred with oedema and cell infiltration in the stroma together with functional but not inflammatory degeneration of the gland. Cystic endometritis is observed during all the phases except early proliferative phase.

#### Fungal infection: (Fusario toxicosis)

When maize infected with *Fusarium graminea* is fed to the animals, on account of its zearalenon ( $F_2$  toxin), which is having oestrogenic effect, produces reproductive problems.

Severity of the problem varies according to the degree of infection and duration of feeding. Besides gastrointestinal symptoms oedema of vulva, anoestrous condition, abortions and malformations of the foetus have been described. Swelling of the udder and teats are markedly observed not only in sows but also in gilts. In male piglets swelling of prepuce also occurs. Younger animals are more susceptible and even with little quantity show severe symptoms, leading to sterility. In sows chances of recovery are remote (Pajusik, 1973; Rudnai, 1974; Nyomi *et al.*, 1974).

Some can be experimentally, by feed...

Such cases stimulate chronic oestrogenic effect such as early maturity in gilts with increased number of Graafian follicles and hyperplastic endometrium.

In sows hyperplasia of endometrium is also markedly observed. Besides increased follicular activity, degenerative processes of the follicular epithelium and ova also begin resulting in ovarian sclerosis. Together with this, cystic glandular degeneration and desquamation of superficial endometrial epithelium also occurs. This is followed by intense cellular infiltration of the endometrium. The hyperleucocytosis is characteristically observed in vaginal discharge. The hypophysis cerebri shows a decline in basophil cells and a great increase in acidophils which suggests that oestrogenic effect is not only peripheral but involves the central regulatory system.

#### Post weaning fertility and infertility

Some sows exhibit oestrus 72 hours after farrowing which is unovulatory (Du Meznil du Buisson *et al*, 1970). During this early oestrus, release of LH cannot be demonstrated (Parvizi *et al*, 1975).

The lactational anoestrus occurs in sows as a result of disproportionate levels of prolactin and gonadotrophins. Immediately after farrowing the level of FSH is high and L.H. level is low. The levels become normal only after weaning (Crigton and Lamming, 1969).

Gonadotrophin inhibition becomes weaker in late lactation, by 40 days after farrowing when spontaneous oestrous cycles may commence. In early lactation (before 30th day) it is hardly possible to induce oestrus with exogenous gonadotrophins. In the later part of lactation, the number of individuals

responding to gonadotrophins increases proportionately as period of lactation advances (du Meznil du Buisson, 1970).

Oestrous cycles usually commence after weaning. Resumption of gonadotrophin function becomes more marked if early weaning is practiced before 30-35th day of lactation. This may be due to rebound effect of prolactin activity which is highest during peak of lactation. As such resumption of sexual functions depend primarily upon the time of weaning (Becze, 1976).

In case of conventional piglet rearing where the suckling period is longer than 40 days the time of the first oestrus after weaning varies considerably (Cole and Cupps, 1959). If weaning is practised on 28th to 30th days, 85% of the sows exhibit oestrus within the first 10 days. About half of the anoestrous sows can be expected to exhibit oestrus within 50 days of weaning, if not they are fit to be culled. If some sows are weaned during the first 4 days of lactation, oestrous invariably occurs within 4 to 5 days following weaning. This is followed by another oestrus after 4 to 5 days which brings sows into normal cycle (Wekerle, 1974). Group weaning is desirable as the endocrine status of each member of the group will be relatively similar which acts as the basis for planned breeding.

Sows which are weaned on the 4th day after farrowing exhibit first oestrus very early. On account of epitheliochorial placenta a very rapid involution of uterus occurs resulting in conception even 2 weeks after farrowing. However, in such cases conception rates and litter size are extremely small. This is partly due to low number of ovulations and partly because of the incomplete uterine involution. Following short lactations as that of 40 days, conceptions are poor



(Gruver *et al*, 1972). Reduced litter sizes after early weaning (before 20th day) have been reported (Parmer *et al*, 1965; Moody *et al*, 1966, 1967 and Groves *et al*, 1967).

The relationship between lactation and uterine involution is manifested as follows, the return of the uterus to its pregravid size takes 3 weeks and the process of involution is very rapid during the first few days after farrowing. In lactating animals the involution of uterus is intense and faster compared to non-lactating ones. In lactating animals, after the involution is completed the endometrium becomes definitely thin. In non-lactating animals and in sows weaned very early, the oedema of the uterus, the infoldings of the endometrium, the development of the glands and strong vascularisation all show the beginning of a new proliferation already taking place on the 12th day post partum. All these changes are related in non-lactating animals to the beginning of gonadotrophic and ovarian activity after the 10th day. These facts also reveal that in non-lactating sows the LH content of the hypophysis is higher between 6th to 11th day. As such the endometrium avoids total involution and starts proliferation early. There is progressive regeneration of uterine wall upto 21st day along-with increased enzymic activity due to proteolytic process which act as barrier for trans-uterine migration, implantation and embryonic development (Thume, 1968; Smidt *et al*, 1969).

#### Anoestrous condition

Reproductive ability tends to become sluggish following farrowing. In certain number of animals (10 to 15%) after a few normal cycles, prolonged anoestrous condition may develop. Prolonged ano-

estrous condition occurring after weaning in lactating animals can be attributed to poor condition of the animal. This form of subfertility causes considerable losses unless early pregnancy diagnosis is undertaken. Early pregnancy can be diagnosed by the use of hormones which will not have adverse effect on the conceptus but may induce oestrus in empty animals. The early pregnancy diagnosis can be done by progesterone estimation (19th day following insemination). The use of hormones is limited in commercial units on account of "Hormonopathia" (missing hormonal effect) which develops mainly against gonadal steroids but to a lesser degree against gonadotropins. Season and strong and short stress especially due to transport play an important role in inducing oestrus.

In order to find out the pathomechanism of this condition Bezce (1976) carried out early pregnancy diagnosis (28-36 day non-returns) with ultra sonic apparatus and with Dopplerechographic method on 1400 sows. Of these 200 (14%) were diagnosed as non-pregnant. During 100 day observation period, 116 out of 200 (58%) exhibited oestrus and 84 out of 200 (42%) were anoestrous. Out of 116 animals that exhibited oestrus 76 became pregnant and rest 10 remained empty. The total number of anoestrous animals being 124 (62%).

Further investigation on 64 anoestrous sows on post-mortem examination revealed following conditions. (Table 32).

Histological appearance of the endometrium was in accordance with ovarian condition.

The incidence of the anoestrous condition varied according to the size of the pig unit and it is the most common cause of infertility encountered in large

Table 32  
POST MORTEM CONDITIONS ENCOUNTERED IN 64 ANOESTRUS SOWS

Condition	No of cases	Description
Infantilism	5	Infantile uterus and small ovaries
Inactive	7	No CL and G F Shape and size of ovary normal
Subactive	13	Regressing CL's 10 15, Lentil sized follicles
Persistent corpus luteum	16	Fully developed CL
Silent oestrus	4	—
Ovarian cysts	22	Single, multiple, large, small with or without persisting CL

units. However, majority of cases respond to improved environmental and managerial conditions viz proper transportation, regrouping of herds, reorganization of the routine schedule and adequate nutrition. This indicates that there is a close relationship between the environmental factors and the endocrine constitution, a small disadvantageous effect induces anoestrous condition whereas a small advantageous effect quickly restores it. The incidence of this transient form of subfertility due to anoestrous condition is about 40% and 1/3 of the cases start exhibiting oestrus after an interval of 1 to 3 cycles without remedial measures. The other 1/3 of the lot requires 2 to 3 months for spontaneous recovery. The remaining 1/3 of the cases can be considered as having ovarian dysfunction and gross endometrial changes.

#### Disturbances in fertilization and influence thereof on conception rate

In the normal course, practically all ova are usually fertilized (90% to 100%, Smidt, 1971) except the abnormal ones like oval, leuiform, with rimose zona

pellucida. In swine, the conception rate determined on the basis of cleavage is slightly lower because of the phenomenon that one part of nonfertilized ova — a cytoplasmic fragmentation occurs very similar to the normal cleavage. These cases can be identified only by intensive examination, since the presence of spermatozoa in the zona pellucida is not a proof of fertilization. Considering this in swine following a well timed insemination with good quality semen the apparent cent per cent fertilization is only to the extent of 85% to 95% (Nalbandov, 1961).

Rate of conception is greatly influenced by the boar. Litter size differs to a great extent from boar to boar and this is mainly related to the individual differences in semen quality. There are cases, where in spite of good quality semen conception rate is poor (Aamdal, 1967).

In the swine on account of the long duration of estrus, ageing of gametes occur resulting in the decline of the conception rate and the rate of embryonic development, in consequence of which the sows lose their ability to

produce fully viable embryos. Later, the number of abnormal conceptions increase due to polyspermia and digynia. If inseminations are performed 36 hours after the onset of oestrus the number of zygotes with more than two pronuclei will increase proportionate in relation to the delay (Hancock, 1959, 1961; Thibault, 1959; Bomsel-Hel Mrich, 1962).

Polyspermia is more common in aged ova since zonal reaction becomes slower. Digynia develops in case the second polar body is not shed following sperm penetration and its chromosome set becomes female pronucleus. This can happen even after well timed insemination but incidence becomes more frequent with delayed inseminations. This indicates that the spermatozoa is not able to induce the final phase of the meiosis (of ovum). According to Thibault (1959) digynia is more frequent than polyspermia.

Ageing of the spermatozoa and the process in which they become unsuitable for fertilization, develop in the genital tract when insemination takes place too early or during in-vitro storage of the semen. Following insemination with semen stored for more than 3 days, a much lower number of spermatozoa can be found in the zona-pellucida and the number of embryos with reduced viability increases (Dziuk and Henshaw, 1958; First *et al*, 1963). It may be assumed that the ageing of the spermatozoa is related to the decrease of their DNA content.

Most of the zygotes from abnormal fertilizations do not develop, others die during the embryonic development and heteroploid offsprings seldom develop from such zygotes. Litter size depends mostly on the rate of embryonic deve-

lopment which in turn depends on the early embryonic losses. Embryonic mortality during nidation is about 25% in multiparous species. This makes possible the elimination of embryos from abnormal fertilizations and of those with reduced viability and the uterus is protected from over-loading. It can therefore be considered as a physiological phenomenon. When embryonic losses are in excess it becomes pathological.

Embryos develop from about 80% of the ova shed, the rest will die before implantation and an additional 3% upto the 45th day of gestation (Becze, 1971).

The causes of embryonic losses are multiple. It is related first of all to those embryos which are from abnormal fertilization and to those which are loaded with chromosomal aberrations. The reduced viability of the embryos formed out of the aged gametes partly originate from chromosomal abnormalities. As a consequence of immune incompatibility the embryos die at the time of implantation.

Genetical factors are probably involved in some boars as greater differences can be found between the rate of fertilization and litter size (Aamdal, 1967; Hafez, 1967). Embryonic mortality is higher when inbreeding is practised and is related rather to the inbreeding of the dams than to that of the foetuses.

Contrary to other species, rate of embryonic mortality increases with increase in age of the sow; it is lower in gilts than in sows which have had many farrowings, though the number of eggs shed is higher in older animals. Rate of embryonic losses is also higher in very young just matured mothers. The proportion of dead foetuses increases after

the sixth pregnancy (Vandeplasseche, 1968).

The physiological capacity of the uterus is also one of the factors of embryonic losses. The rate of embryonic loss increases with the increase in number of ovulations (Hammond, 1927 and Nalbandov, 1964). This does not mean however that higher ovulation values are not desirable because the higher litter size depends on higher rate of ovulation.

The average value of physiological capacity of uterus in sows is about eighteen embryos. Following transportation of blastocysts embryonic losses increase as both the recipients, ovum and the transplanted blastocyst might be destroyed (Vandeplasseche, 1968). Even in spite of the fact that superovulation was induced by PMSG administration (1500 I.U. PMSG on the 15th day of the cycle) and 35.4 ova obtained only 17 live embryos developed limited to the physiological capacity of the uterus (Hunter, 1966). Limitations of uterine capacity might be related to the placental conditions required for nourishing the foetuses. When embryonic losses occur, foetuses located at the cornual apices are mainly affected. The relationship between nutritional status and embryonic loss is as follows: Increased caloric values of the sows feed increases the number of ovulations, the size and weight of the corpora lutea and their progesterone contents. In such cases the number of embryos increases even beyond the physiological limits. When the number of embryos exceeds the uterine capacity, the magnitude of the embryonic deaths will be so great that the expected size of the litter decreases. Therefore reducing the rations following insemination is advisable if flushing has been practised to stimulate

ovulation (Nalbandov, 1964; Vandeplasseche, 1968).

Decreasing the caloric content of the ration results in increased embryonic losses. If the required portion is supplied in the form of maize 46 days before insemination, the rate of ovulation remains unchanged but only 22% of ova will develop into embryos, instead of expected 30%. If the same treatment is applied following mating, embryonic losses before implantation will be increased from 2-3% to 5% (Becze, 1962; Hafez, 1967). Very little is known about the effect of thermal stress on embryonic survival but litter size is increased when sows are protected against summer heat by water spraying (Hafez, 1967).

Little is known about the hormonal play in embryonic deaths. Litter size was not increased following progesterone treatment (Becze, 1967) or oestrogen (Day *et al*, 1963) treatment applied during implantation. Hormonal treatments (oestrogens + androgens or gonadotropins in low doses) which are used for early pregnancy diagnosis do not affect litter size. Considering the above it can be concluded that any litter size below the average of 10 piglets should be considered as indicative of higher embryonic losses.

Embryonic loss of about 20-30% can be judged by comparing the number of corpora lutea of the live embryos at about the 45th day of pregnancy (Becze, 1969). If the number of the ovulated follicles (17) is taken as a basis, embryonic losses amount to 41%. If litter size is related to the number of follicles suitable for ovulation, which is 19, few hours before ovulation, the loss is 48% (Nguyen Thien, 1974). The above studies indicate that in swine polyovulatory follicles with more than one ovum can be encountered (Becze, 1969).

### Perinatal losses, perinatal diseases of the genital tract

Perinatal losses are characterised by the number of foetuses born dead-stillbirths. In commercial herds, incidence has gone up from 5.8% to 15-20%. Over 30 factors have been attributed to the probable cause of stillbirths. It has been observed that in 30-50% of the farrowings, foetuses are born with ruptured umbilical cords resulting into stillbirths (Perjes, 1976). If the umbilical cord ruptures and if the farrowing is delayed due to uterine inertia, the foetus dies of suffocation. The cause of this rupture was earlier considered to be of genetical origin but it is now considered to be the consequence of environmental factor, delayed farrowing and decreased intensity of the farrowing. The newborn foetus should not be separated from the mother, she becomes nervous and if presented later she develops aversion. However the foetus will remain under her supervision. The newborn beneath the drier lamp starts suckling soon and induces favourable oxytocin release in the quiet sow. This ultimately produces favourable effect on the rhythm and intensity of farrowing.

Increasing number of piglets having low body weight in commercial herds is another important cause. In some cases, large foetuses result into dystokia and consequent death of the foetus. The etiological conditions of above are not clearly known.

In consequence to large sized foetuses decreased number of foetuses develop in the uterus. In case of sows with few foetuses in uterus the nutrition helps in increased development of foetus leading to dystokia. Lack of exercise can be an important factor in causing dystokia alone but its effect is increasingly

harmful when there are few foetuses in uterus getting more nutrition. Litter size decreases due to early breeding, fungal infections of feeds and early embryonic deaths. This is more common in gilts. Sows if bred within 20 days after farrowing have decreased litter size due to incomplete involution of the uterus.

Little is known about the causes of low birth weight and nonviable piglets. In such cases in spite of normal number of ovulations, fertilization and nidation, the uterine environment is not conducive to the growth of foetus. This may be attributed to altered hormonal status (Hormonal apathia).

### Mastitis, Metritis, Agalactia (MMA-Syndrome)

MMA syndrome leads to heavy losses by increasing neo-natal mortality and by delaying conception after farrowing. The syndrome has been first reported in U.S.A. in the fifties. The incidence of this condition in commercial herds is about 15-20% and is the most important reproductive problem in France (Wilmore, 1974). The syndrome becomes apparent 12-48 hours after farrowing commonly involving the mammary glands and seasonally the genital tract. Sometimes arthritis is also encountered. Constipation is the general symptom. On account of agalactia and mastitis, sows lie prostrate and suckling is suspended. The mammary glands are oedematous and inflammatory. Milk with apparently normal consistency having offensive smell is discharged from some of the teats. Scarlatinal excretions indicating inflammation are also observed. The mammary gland may show necrotic patches when the genital tract is involved. Serous, mucous, mucopurulent discharge or in severe cases, redish

brownish offensive vaginal discharge is observed

Shreds of foetus or foetuses or placenta may be present in the vagina. Post mortem findings also show inflammation with varying degree of severity. Atony not only of the uterus but also of the alimentary canal is present. It is usually related to delayed farrowings beginning with high temperature and constipation accompanied by muscular weakness and circulatory failure. In some cases in spite of relatively undisturbed general condition, mastitis of one or more quarters and mild endometritis could develop after farrowing. In such cases the normally occurring post partum high body temperature remains at a higher level for weeks (Brenner 1972). *E. coli*, *staphylococcus*, *Klebsiella*, *streptococcus* and *mycoplasma* strains can be isolated from the affected organs and this indicates external infection. Etiology of the infectious agents is however not clear. The MMA syndrome probably is due to endocrine disturbances as a result of stall feeding and inadequate nutrition. Systemic disturbances occur due to lack of exercise over feeding rations causing constipation, damaged grains or fodder and feed containing oestrogenic substances. Sudden environmental changes or very high temperature inside the pens may lead to detrimental changes of the endometrium (Wilmore 1971). High piglet mortality due to this syndrome is of economical significance. During starvation piglets die soon because of hypoglycaemia unless they are artificially reared. Mortality is practically very little in sows as they recover soon from septic endometritis due to antibiotic treatment and rarely develop sterility (Suveges and Vamou 1973, Suveges *et al.*, 1974 and Radnor and Radnaine, 1971).

The three symptoms metritis, mastitis and agalactia do not occur together in every case, can develop one by one or only two of them may occur at one and the same time (Becze, 1975). Lack of free movement before farrowing, housing without bedding, artificial illumination, increased noise effect, improper ventilation, contribute as predisposing factors for causing retained placenta, metritis and delayed involution. Crowding of sows in small rooms before farrowing is not advisable. This unnatural housing will induce in a psychosomatic way by lengthening of gestational period from 3 to 5 days, delayed farrowing, dystokia, retention of foetal membranes and metritis. Under such conditions the object of having farrowings is defeated. If the animals within the unit are provided with more ideal conditions the incidence of disorders will significantly decrease which supports the importance of environmental influence (Becze and Szilvassy, 1976).

### Nutrition

Plane of nutrition has a marked effect on the fertility in the female swine. Inanition (due to low plane of nutrition) in swine results in delay in the onset of puberty and failure of oestrus or irregular oestrus. Over feeding or high feeding levels cause early embryonic mortality in increasing degree (Casida 1953). Narayan Rao (1969) reported on his observations on the swine herd at Tirupati Veterinary College that feeding pigs for prolonged period on garbage i.e. largely on cereal food wastes along with vegetable matter variable in quantity and quality resulted in poor growth rate, late maturity and delayed returns. Robertson *et al.* (1961) reported that the percentage of ova developing to normal embryos at 25

days of gestation was less by 25% in sows on a full feed programme. Christian and Nofziger (1952) estimated the prenatal death rate as 27% greater on a high plane than on low plane of nutrition in the swine. Even though high ovulation rates occurred with gilts on full feed, the higher embryonic death rate produced litter smaller than those in gilts on a low plane of nutrition. Hains *et al* (1955) however observed that although gilts fed on full feed had a higher embryonic death rate than gilts on low feeding levels, the high ovulation rate resulted in more pigs farrowed. Casida (1953) showed that full feeding exerted its effect during conception and soon after and was not related to the same condition at the time of breeding. Moustgaard (1959) reported that feeding sows on plant protein without Vitamin B12 caused a higher embryonic death rate than in sows which were fed animal protein or plant protein with Vitamin B12.

Vitamin A deficiency in swine causes nervous symptoms, irregular oestrous cycle, frequent and longer duration of oestrus and lower conception rate. Hughes *et al* (1928) observed that gilts bred before nervous symptoms developed usually either aborted or farrowed dead foetuses or the piglets were weak at birth and died soon after. Resorption of the foetuses may occur in some sows. Hale (1935) Bendixen (1944) and Bauer (1950) reported the presence in new born piglets of embryonic defects and abnormalities caused by Vitamin A deficiency in sows during early pregnancy. The anomalies included eye defects or the absence of eyes.

Swine are the only farm animals in which deficiency of calcium may affect reproduction. Evans (1929) reported that if pregnant sows are fed on ration

deficient in calcium it may cause difficulties in farrowing, low milk yield and the piglets which would survive are apt to be weak and unthrifty. Davidson (1930) reported that sows developing severe calcium deficiency had a tendency towards an increase in foetal atrophy and still births.

### Infectious infertility

Normally the genital tract of sows is clean owing to the self purificating ability of uterus and vagina especially during the follicular phase. During farrowing, the genital tract often gets contaminated with nonspecific or facultative pathogenic microbes. They rarely induce a chronic catarrhal endometritis, however the common organisms producing mild endometritis were isolated from the genital tract of repeat breeder sows. Chronic infections in the urogenital tract are caused by some facultative species like *E. coli*, streptococcus and staphylococcus. These strains will reach biological equilibrium. In such cases when one of the organisms is eliminated by treatment, the eco-system becomes unbalanced and the other non-specific organisms proliferate thereby making recovery impossible (Brenner, 1972). The atony of the uterus coupled with decreased resistance of the sow is responsible for increased virulence of the microorganisms involved.

### Brucellosis

Brucellosis is the specific infectious disease caused by *B. suis* and in some countries by *B. melitensis*. *B. abortus* even in massive infections only causes serological changes. Transmission of Brucellosis is mostly due to ingestion but it often takes place venereally through infected bears or their semen.

Dermal infection should also be taken into account. Intra-uterine infection has an important role in maintaining the disease. *Brucella* infection spreads quickly in the sow causing pathological changes in different organs such as abscesses, deformity of the vertebral column, by development of exostosis which results in paralysis as a consequence of pressure on the spinal cord. *Brucella* organisms have a special affinity towards both the male and female reproductive organs, foetal membranes and foetus.

In boars, orchitis at times breaks out in an epidemic form. There will be swelling of the testis which is not painful and the libido decreases. In the later stages sclerosis of the testis occurs. The boar testes are relatively rarely involved. Epididymis and seminal vesicles are most frequently affected while as the prostate and cowpers glands are rarely involved. In testis and accessory glands lesions are not apparently visible but can become evident microscopically. Although these foci reveal degenerative changes, they do not develop into abscesses. The inflammatory changes obstruct the efferent ducts of the testis which inhibit the appearance of leucocytes in the semen ejaculate of the boar in spite of the fact that the *brucella* organisms appear in greater numbers at this site. There is no continuous discharge of *brucella* organisms, as such at times they are not seen in the ejaculate.

Ejaculates of boars without clinical symptoms are very often good. In case of subclinical affection of testes and epididymis, the number of abnormal spermatozoa increases and in severe seminal vesiculitis, the volume of ejaculate decreases. Good ejaculates are correlated with good fertility though

bacteria are transmitted through semen in every case. However the venereal transmission cannot be ignored.

In sows, *Brucella* organisms prefer to establish in uterus and foetus. Early positive serological titre indicates the generalisation of the infection. Subsequent to infection small necrotic foci develop in the wall of the uterus, unaccompanied by any discharge. Infection of uterus does not interfere with pregnancy but influences its normal outcome since the infection induces exudative inflammation of foetal membranes and the foetus becomes infected resulting in abortion. The stage at which abortion occurs depends upon the time of infection, mostly between 2nd to 12th weeks or even earlier but seldom later. Early abortions go undetected. Later abortions are characterised by general drowsiness and cardinal symptoms of abortions occur. Abortions usually have a smooth course. Sometimes it is followed by retention of foetal membranes or followed by mucopurulent vaginal discharge with shreds lasting for 8 to 10 days. Severe metritis may cause sterility. In case of recent infection, pregnancy may or may not be terminated. In such cases, the sow besides viable piglets may give birth to the ones which had died earlier at various intervals and become macerated and mummified as well as to piglets with decreased viability and delayed development. This occurs because the foetuses develop in well separated ampullae of the uterus and the pathological processes are localised. Consequently some foetuses may get infected at various stages of gestation whereas others remain unaffected.

The uterus gets rid of infection soon after farrowing and there is early resumption of normal oestrus and concep-



tion. A certain degree of immunity develops after the primary infection in consequence of which subsequent abortions are seldom. In the infected herds however, lower conception rates are expected if early inseminations are done soon after abortion. Late return to service is more frequent because of increased embryonic mortality and litter size is also decreased.

Number of piglets born dead and those with reduced viability is increased, the latter ones will die during the rearing period because of infection. In case of infected sows usually the third pregnancy is normal again.

In brucellosis, antibiotic therapy is ineffective. Eradication of the disease can be done as follows:

The infected units are considered as 'closed herds', boars with clinical symptoms of brucellosis are culled, sows which are aborted are separated and use of infected breeding material is avoided. It is difficult to carry out the above suggested practice. Slaughtering of the infected herd seems to be the only effective method. It is difficult to establish non-infected herds because even the repeated serological tests will be positive in few animals in the apparently non-infected herd. Animals for breeding can be purchased from those places where the disease has not occurred for a long time, neither in the herd nor in its vicinity (Vandeplasseche *et al*, 1967 and Roberts, 1971).

### Leptospirosis

Leptospirosis does not cause infertility but may cause mass abortions in sows. *Leptospira pomona* and *L. hyos* are the most common strains like (Manninger, 1959) and the other strains like *L. haemorrhagica*, *L. canicola*, *L.*

*grippotyphosa*, *L. hardjo*, *L. serjoe*, (Roberts, 1971) are of less importance.

The causative organism infects the swine through several hosts. The infected pigs void *Leptospira* for a long period and thus the disease spreads in the same unit as well from unit to unit. Infection spreads through urine, coitus and semen of infected boars. In the initial stages only high body temperature is observed, seldom accompanied by encephalitis or nephritis. However sows in their second half of pregnancy will abort or give birth to non-viable piglets. Early infection does not endanger the pregnancy, but infection during second half is harmful to foetuses. Infected sows develop lifelong immunity. The character of abortions and changes in the foetus are similar to those in brucellosis. Serological tests should be conducted for differential diagnosis. Even with apparent recovery, the affected sows remain as carriers and keep on voiding organisms for about a year and are dangerous to animals which are immune and to those in advanced pregnancy.

In order to prevent further losses due to abortions, feeding of tetracycline @ 500 gm/1 ton. of feed for 2 weeks is recommended (Manninger, 1959 and Roberts, 1971).

Prevention of the disease is possible by building up clean herd based on serological tests. Animals ones infected remain sero positive for three years though they may void *Leptospira* for only one year after recovery. Prevention of disease is possible by vaccination if non-infected young animals are vaccinated immediately after breeding and revaccinating them in early pregnancy. Immunity by vaccination is of short duration (Kemenes, 1971). Piglet infection can be prevented by early

weaning and by their separation from infected stock

Wide variety of micro organisms induce embryonic mortality and abortions, occasionally in the form of local epidemics. The same holds true for viral infectious swine pest, Ajuszky disease, Picorna virus, enterovirus and Parvo virus SMEDI virus. Preventive vaccinations against swine pest by using inactivated or attenuated or lepinised vaccine in early pregnancy causes embryonic deaths or malformations

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# Chapter 27

## Infertility in the Bitch and Cat

### Anatomical or congenital defects

These are of rare occurrence in female dogs and cats. Lee and Allum, (1952) and Leonard *et al* (1953) described cases of true hemaphroditism in the dog and cat. It is characterised in the dog by having an abnormally large clitoris. These animals show abnormal sex desire and activity until they are spayed.

Sweet and Martin (1955) observed three cases of uterus unicornis in unrelated dogs in approximately 2000 spaying operations. Stephenson and Leonard (1954) reported only one case in every 10,000 dogs. They also observed uterus unicornis in three cats only. Sheppard (1951) reported 21 cases of uterus unicornis or an incidence of

between the ovary and uterine horn in the dog. The condition was rare in the cat. The remnants may be parametrial, occasionally para ovarian and rarely myometrial or in the fimbriated extremity of the oviduct. Hypoplastic or missing ovaries are rare in dogs. Cysts of the Gartner's duct in dogs and cats and Bartholin's glands in the cat are rare. Tortoise-shell or black and yellow cats are invariably females (Bloom, 1953).

### Nutritional causes

Severe emaciation or inanition will cause delayed puberty or failure of oestrus. Obese female dogs or cats are likely to be sterile due to endocrine disturbances, causing irregularities in oestrous cycle.

is the only treatment for such a condition. In dogs and cats, it is commonly observed that, there is increased incidence of ovarian cyst with age. Bloom (1953) reported that nearly 90% of aged female dogs had ovarian cysts. Cysts of a diameter of 0.5 cm. to 6 cm. may develop from atretic follicles or mature follicles. Cysts which are formed from atretic follicles, there is absence of hyperestrinism. Cysts developed from mature follicles with a well established granulosa and theca interna layers are rich in oestrogens. In aged dogs hyperestrinism is invariably accompanied by varying degrees of endometrial hyperplasia. The cystic endometritis may cause hydrometra, atrophy and fibrosis of the uterine wall, which predisposes the uterus to various infections resulting in metritis and pyometra. Hyperestrinism causes swelling of the vulva, alopecia, mammary and uterine tumours, abnormal uterine bleeding during proestrus, irregularities of the oestrous cycle, possible dangers of abortion, infertility and sterility.

Hypothyroidism in cats may be due to absence of thyroid gland or a prolonged insufficiency of iodine in the feed leading to atrophy of the thyroid gland (Scott *et al*, 1961). The affected cats are stunted, have sparse coat, thickened skin and the head looks broad due to edema. Their movements are sluggish. In prolonged deficiency there is loss of sexual activity. However queens which are on the border line of deficiency may conceive and carry the foetuses to term or the gestation may be prolonged. Parturition in such cases is slow and difficult and the kittens have congenital deformities such as open eyes and cleft palate (Scott and Humphrey, cited by Catcott, 1961).

### Anoestrous condition

This condition is commonly observed in one to two year old bitches even though they apparently look healthy. In these bitches, the ovaries are small, hypoplastic and may be juvenile (Benesch and Wright, 1951). Treatment of such young bitches with anoestrous condition is not successful. If the anoestrus condition is due to inanition, proper feeding is found beneficial. Scrogie (1939) reported 18 bitches with abnormally prolonged anoestrous periods and obtained beneficial results after administration of relatively small doses of gonadotropins. Hancock and Rowlands (1949) reported discouraging results with PMS and stilboestrol. Bloom (1953) reported that cystic corpora lutea are very rare in dogs and cats.

### Nymphomania

This condition is characterised in the bitch by intense sexual desire which becomes evident when the bitches start mounting the male dogs. In the usual course the affected bitch will not permit copulation. The vulva is swollen and oedematous and bloody discharge is commonly observed. In most cases it leads to sterility. There is marked nervousness further developing in to vicious condition. Multiple follicular cysts appear in ovaries and these are thin walled and of about 2.5 cm. in diameter. Due to prolonged estrogenic stimulation, the uterine endometrium becomes hyperplastic and cystic. Cats with cystic ovaries are sterile. Nervousness, timidity, occasional fits and skin troubles are the most common symptoms in nymphomaniac cats. In cats when the cystic ovaries become very much enlarged, the ovaries can be palpated behind the kidneys through

the abdominal wall. No cure is possible even if the cysts are ruptured. Ovariectomy is the only satisfactory way to overcome the condition.

Perpetual oestrus may be observed in bitches with cystic ovaries and in spayed females in which ovarian tissue is not properly removed. Metritis and uterine tumours are the other causes for constant oestrus. Care should therefore be taken that when bitches are spayed, the ovaries, uterus and cervix should be removed properly. Removal of the anal glands is also indicated.

In cats diagnosis is likely to be faulty since they make considerable fuss during oestrus, when they are likely to be diagnosed as nymphomaniac.

According to Bloom (1953) insufficient secretion of progesterone may result in sterility and cause loss of embryos or foetuses in bitches. He therefore advised administration of progesterone from the time the oestrus has subsided until the 8th week of pregnancy. In bitches showing abnormally long oestrus period, it is possible to hasten ovulation by injections of chorionic gonadotropin.

of estrogens and testosterone as treatment for pseudocyesis that usually occurs late in the metoestrous period. Ovariectomy will prevent its occurrence. However most bitches will recover without any treatment, since this is a normal physiological process, oestrogen administration for prolonged periods may cause ovarian cysts and changes in the endometrium.

### Preventing conception in bitches

A number of bitches are usually offered to the veterinarian for prevention of conception after they are accidentally mated. Administration of Diethyl stilboestrol in the dose of 2 mg. per kg. body weight gives fairly good results, if treatment is done within 24 to 48 hours after undesired mating. The injection should be repeated on the third day. Use of stilboestrol may however result in prolonged oestrus and cystic ovaries.

### Incontinence of urine

This is common in spayed bitches particularly marked in larger breeds. The dribbling of urine is due to insufficiency of estrogen which causes lack of tone in the sphincter muscles of urinary tract. Administration of stilboestrol is found beneficial to correct this condition.

## Specific infections of the genital tract

Tuberculosis of the uterus in the dog and cat are rare (Bloom, 1953). Pathology of the genital tract in dogs and cats is mainly due to two conditions (1) neoplasms (2) infections.

### Neoplasms or tumours of genital tract

These are common in dog and rare in cat. The following type of tumours have been encountered viz. cystadenocarcinoma, luteoma, dysgerminoma, theca cell tumour, fibroadenoma, carcinoma, cystoma, myxosarcoma, teratoma, fibroma, adenoma, sarcoma, cystadenoma, leiomyoma, lipoma, chorio epithelioma, angioma, lipofibromyoma, lymphoblastoma and transmissible venereal tumours.

Uterine and ovarian tumours can successfully be removed by laparotomy followed by ovario hysterectomy. However prognosis would be poor in case there is metastasis. Prognosis is also uncertain in the presence of pyometra. Many of the vaginal and vulvar tumours are pedunculated and can be removed with ease by ligating the pedicle. If the base of the tumour is broad, a dorsal episiotomy is indicated in order to obtain a clear exposure of the vagina and tumour, so that complete removal can be accomplished.

### Infections

Infective inflammatory conditions of the canine female genital tract are quite commonly met with.

#### Ovaritis — oophoritis

This is of rare occurrence in the dog and cat, even when associated with pyometra (Bloom, 1951).

### Acute metritis and vaginitis

This may occur as a result of difficult parturition, dystokia, emphysematous condition of the foetus, contusions, lacerations of the birth canal and uterus, retained placenta, abortion and delayed uterine involution. All these conditions are generally characterised by fever, anorexia, drop in the milk yield, depression, a foetid bloody discharge and other symptoms associated with an acute septic metritis. Acute metritis may develop in cats after several abortions (Sheppard, 1951).

### Chronic vaginitis and vulvitis

This may occasionally be observed in the intact or spayed females of any age. The causes are similar to those of vaginitis and vulvitis in other animals i.e. either infection or trauma or both in combination. The infective organisms include Streptococci, Staphylococci, Coliforms, fungus, etc. Debilitating diseases may predispose the bitch to vaginitis (Bloom, 1954). It is rare to find vaginitis in a spayed bitch, secondary to fistula from the ligature around the stump of the vagina or cervix in consequence of the use of non-absorbable sutures. Treatment with antibiotics may give beneficial results. If the fistula is due to ligature, it should be removed.

### Chronic or subacute metritis or endometritis

This condition is most commonly seen in bitches and cats which are over 6 years of age. It is commonly associated with diffused endometrial hyperplasia due to prolonged oestrogenic stimulation secondary to ovarian cyst. Uterine lesions observed in this condition are oedema, haemorrhage, diffuse leucocytic infiltration of the endometrium, fibrosis



of the uterine wall and occasionally ulceration, abscess formation, necrosis and thrombosis. The condition may be associated with irregular oestrous cycles. Chronic endometritis commonly becomes more acute during the first half of metoestrus or the luteal phase of the cycle. However metritis can develop and produce clinical symptoms at any stage of the oestrous cycle. In the acute stage the vaginal discharge is persistent, profuse, bloody and purulent. This may be associated with anorexia, slight elevation of the temperature, depression, increased thirst and leucocytosis. After the acute stage has subsided, no symptoms may be observed except occasional voidance of vaginal discharge and little loss of condition.

Exacerbation of chronic metritis during metoestrus may result in pyometra. Bitches and cats having a low grade endometritis may fail to conceive or even if conception occurs, it may result in abortion. The litter size may be small, the pups or kittens are dead or macerated, or they may be weak ones and die within few hours or days after birth. Leonard *et al.* (1953) pointed out that such a condition should not be confused with neonatal isoerythrolysis in which the young ones are born strong but succumb several days after they begin to suckle. Spaulding cited by Roberts (1956) pointed out that in cats with chronic endometritis, a progressively smaller litter size, an increased difficulty in conceiving and seldom survival of the new born are the conspicuous symptoms. Subacute metritis was observed more in aged cats. In such a condition there was fibrous proliferation of connective tissue in the uterine wall. Material from such cases subjected to cultural examinations showed the

usual pathogens, such as *Streptococci*, *Staphylococci*, *Coliformi* and others.

Prognosis is usually poor owing to frequent relapses and complete recovery is very seldom. Antibiotics may prove beneficial if administered during oestrus; metoestrus and following parturition. Oestrogen therapy may prove beneficial in the metoestrous stage. However the therapy will only be effective in case there is no cystic endometrial hyperplasia. If oestrogens are used in the presence of cystic endometrial hyperplasia, there is every danger that the condition may aggravate. Ovario-hysterectomy is the best permanent treatment.

#### Lesions of the oviduct

These are rare in bitches and cats. Pyosalpinx is usually secondary to pyometra (Bloom, 1954).

#### Pyometra

This is one of the most common pathological condition affecting genital tract in bitches, but less commonly observed in cats. The aetiological factors are similar in dogs and cats. The condition is observed more frequently in the age group of six years or above. Benesch and Wright (1951) reported that the incidence was more in nulliparous than in pluriparous bitches. Enforced prevention of conception for years may predispose bitches to cystic ovaries and hydrometra.

Pyometra is characterised by an accumulation of pus in the uterus on account of the closure of the cervix due to anatomic malformation or a functional failure of the cervix. This is invariably associated with preceding inflammatory condition of the uterus (Bloom, 1954). In this condition both

uterine horns are usually involved. In very rare cases, however, one horn may be found affected (Hogg and Holroyd, 1955).

### **Etiology**

It is generally accepted that most cases of pyometra in the bitch are preceded by an endometrial hyperplasia due to excessive secretion of oestrogens by the cystic ovary. Ovarian tumours including granulosa cell tumours and papillary cyst adenocarcinomas may also produce the "Swiss cheese" type endometrial hyperplasia (DeVita, 1939; McEntee and Zepp, 1953; Bloom, 1954). Development of uterine tumours (adenomyosis) may block the lumen of uterus and prevent escape of the uterine exudate. Atresia of the genital tract, congenital or acquired, as well as fibrosis or oedema of the cervix may obstruct expulsion of uterine exudate. Increased uterine exudate of chronic endometritis, which develops under the influence of progesterone during the metoestrous stage may act as contributory factor in developing this condition. During the luteal phase, the cervix tends to close but the uterus is relaxed (Erikson, 1952; Benesch and Wright, 1951, and Teunissen, 1952). Pyometra develops most commonly during the metoestrous or pseudopregnant period. Pyometra following parturition or abortion is seldom observed in the bitch. When resistance is lowered during luteal phase infection can flare up and lead to pyometra. Pyometra is usually secondary to endocrine imbalance. If infection is present in the uterus at a time when there are extensive cystic endometrial changes it can not be overcome during oestrus readily as with normal uterus. Thus during metoestrous stage the infection and mucus present in the

uterine cavity and the relaxation of the uterus provides an excellent opportunity for bacterial growth to occur and develop into pyometra. In the etiological factors, endocrine changes are of primary importance and infections are secondary.

Teunissen (1952) recorded that nearly 50% of the infections in canine pyometra were due to *E. coli*. Streptococci, Staphylococci, *Proteus* and other organisms form rest of the 50%. Hughes (1939) recovered both haemolytic and non haemolytic organisms. He showed that infection of uterus may occur from haematogenous and lymphogenous sources as well as through the genital tract.

Injections of large doses of oestrogens to prevent conception or to overcome pseudo pregnancy in bitches may predispose to endocrine upsets and result in pyometra.

### **Pathology**

In pyometra there is varying degree of cystic hyperplasia of the endometrial glands. Leucocytic infiltration of the endometrium also occurs in varying degrees. The cystic glands may or may not be found filled with exudate. In most of the cases, one horn may be found more severely affected. A certain portion of the same horn may be found involved to a greater extent. In advanced cases, necrosis of the endometrium may be present. The myometrium may show evidence of fibrosis and invasion by endometrial glands. The ovaries usually contain cystic follicles or tumours. Corpora lutea are usually found undergoing regressive changes (Leonard *et al*, 1953; Bloom, 1954). Occasionally pyometra may occur without endometrial hyperplasia or preceding inflammation.

## Symptomatology

In the bitch pyometra mostly develops from 2 to 8 weeks after oestrus. There is pear shaped enlargement of the abdomen. In many bitches there is history of irregular or frequent oestrus or nymphomaniac behaviour. The vulva is swollen and hypertrophic. In certain bitches there is history of repeat breeding. It is necessary to obtain correct history in some bitches if they were spayed or not and if so whether by an ovariectomy. The bitch may show depression of varying degrees. The attitude may however be good. The general condition and hair coat may however be good. The general condition and hair coat may be from good to worse depending upon the intensity of the disease. In acute cases, body temperature may be high. In chronic cases, it may be normal, and in severe toxic cases it may be subnormal. Anorexia, intense thirst, polyurea dehydration and occasional vomiting may occur. There may be no discharge from the vagina or it may be scanty or profuse. The colour of the discharge varies from yellowish grey to reddish brown with a characteristics odour. The degree of dullness or toxicity is dependant upon the severity. In affected cases having closed cervix, toxicity is more than in cases where cervix is relaxed and the discharge is drained. The pulse is usually high and rapid. The mucous membranes may appear pale, and there is anaemia in chronic cases. Distended uterus may be felt by abdominal palpation. The course of pyometra is variable. In some cases it becomes acute within a week or two. In cases with open cervix it may run a course of a month or so. Uterine torsion of one horn associated with pyometra was reported by Bloom (1954). He stated that

in the bitch perimetritis and parametritis may develop occasionally whereas in the cat a purulent peritonitis may occur secondary to pyometra. He reported that remissions in the condition may be frequent in middle or late stage of metoestrus or early anoestrus. No remission occurred in bitches in which the genital tract was obstructed by tumour or in which there was fibrosis or chronic inflammation of the cervix.

## Diagnosis

In the initial stages, this is mainly based on history, symptoms and physical examination. Rectal examination may be made by the index finger after the bitch is made to stand with fore parts raised. The distended uterus can be felt due to the back pressure from the abdomen.

In closed cases of pyometra, radiography may help particularly in small bitches to recognise it by the shadows of the distended cornua.

Haigler and Hawkins (1910) and Khuen *et al*, (1910) reported that in pyometra, the WBC cell count is usually over 18,000 to 20,000 per cm. and may even reach to 60,000 to 100,000. Anaemia may develop in severe prolonged cases. The blood count may help to differentiate between pyometra and metritis as there is a higher concentration of white cells and a greater shift to the left in pyometra.

## Prognosis

In the bitch and cat, prognosis in pyometra is usually poor, since it is mostly impossible to restore fertility. Pyometra is one of the most common cause for death in bitches. The prognosis will vary from fair to worse, depend-

ing on the condition of the bitch, intensity and toxicity. Rehfeld (1954) and Bloom (1954) reported that in many cases, nephritis may be a complicating factor in bitches affected with pyometra.

### Treatment

Treatment with oestrogens and antibiotics, sulphanilamides and antiseptics may show temporary improvement, as relapses invariably occur and as such there is great uncertainty with any type of therapy. Ovariohysterectomy is therefore the treatment of choice and is commonly preferred, since there are hardly any hopes to restore breeding capacity.

Surgical procedures have to be adopted in good time as delay may cause them unfit to withstand surgical intervention.

### Hydrometra and Mucometra

This may occur due to endometrial hyperplasia especially during metoestrus when there is functional closing of the cervix (Bloom, 1954). One or both horns may be involved and the horns are distended with fluid which is clear and watery or it may be yellowish grey in colour. There is no presence of bacteria, red blood cells or leucocytes in this fluid. The uterine wall usually becomes thin and myometrium becomes atrophied. Closure of the cervix may be physiological during metoestrus or it may also be due to atresia, tumours, fibrosis, endometrial hyperplasia or other causes.

Prognosis in most of the cases is very poor. Ovario-hysterectomy is therefore indicated.

### Pyometra in the cat

It is less common in cat as compared to that in dog. Dow (1957 and 1962)

studied 91 natural cases in cats of which 75% were in age group of six or more years. No case was observed in cats below 3 years of age. Of the affected cats whose breeding history was known, 77% were nulliparous. In the majority of parous female cats, the interval between last parturition and the time of examination was at least 3 years. Very few cases were seen during the winter months from November to February, when cats naturally have their anoestrous condition. About 50% of the affected cats lived in tenements and had a history that they were not mated. It was impossible to ascertain the approximate relationship between the onset of pyometra and last oestrus with regard to the interim duration as is clearly manifested in the bitches, although 50% affected cats had been in oestrus within two months of examination. Berthelon cited by Arthur (1959) demonstrated that cases of acute endometritis in cats, recovered following hysterectomy operations. The effect is probably due to the removal of the source of progesterone. Dow (1957 and 1962) considers that cystic hyperplasia pyometra complex in cats has the same aetiology as in the bitch. The glandular hyperplasia of the uterus is due to repeated cyclical effects of oestrogens and progesterone in nulliparous cats. In later stages pyometra with *E. coli* and haemolytic streptococci becomes superimposed during the metoestrous stage.

### Foetal resorption, mummification and abortion

Apart from the infective factors, the possible causes of foetal death in bitches and cats are lethal factors, lack of uterine space, placental haemorrhage and hormone or vitamin deficiency. Freak (1962) suggested that preexisting state of slight cystic endometritis may act as

contributory factor. Wright (1943) noted disparity in size of conceptus in some bitches when palpated for pregnancy at 24 to 30 days. Freak (1962) made similar observations and confirmed that when the disparity was great, the bitch was subsequently found empty or the final number of foetuses at term was low. In two bitches in which foetal units were detected at 30 days and which produced no puppies at term, a blackish red rather tarry, very viscid fluid was discharged from the vagina on the 64th and 70th day respectively. Foetal death beyond 45 days of pregnancy may result in abortion of the whole litter or the individual conceptus may die, become mummified and be carried to term along with the live foetuses. Mummified foetuses are of different sizes, indicating that foetal deaths occurred at different times in the second half of gestation period. If one or two foetuses survive to term, it may cause overgrowth of foetuses, terminating in uterine inertia. Mantovani *et al.* (1961) encountered an outbreak of abortion in bitches in which  $\beta$ -haemolytic streptococci was isolated and afterwards, the cultures were used experimentally to produce the disease.

#### Death among new born puppies

A serious disease known to cause deaths among recently born puppies is reported by dog breeders since long. In this affection the recently delivered bitch appears to be perfectly healthy, has normal parturition and puerperium. Lactation is normal with adequate yield for the new born and yet with all the apparently normal condition, most of the puppies in the litter, die as a rule during the first week of their life. Majority of the death occurred between the first three days. In rare instances, a puppy may survive in spite of severity of

the initial attack but succumbs a few weeks later from subacute enteritis and diarrhoea. It is interesting to note that those which are the largest and fattiest at birth are the first to succumb, while as the weak ones may survive. There are no striking signs of ill health. An apparently normal puppy at night may be found dead the next morning. The affected puppy is cold with harsh coat. Its skin appears as an illfitting jacket. It makes very feeble attempts to suckle and soon ceases to suck. The bitch will push such a feeble puppy into the corner where it dies very quickly. Instances are on record that frequently several or even all the bitches in a breeding kennel lose their litters in this manner. Very often an individual bitch loses a number of litters in succession. However, it is also found that bitches which have lost their litters may rear subsequent litters normally. The disease seldom occurs in the single pet bitch of the household and it is most uncommon in mongrels. Smythe (1928) observed on post-mortems, a blood stained exudate or freshly clotted blood in the peritoneal cavity together with inflammatory condition of intestines in puppies which died within first three days after birth. In cases where death was preceded by illness by several days both pleural and peritoneal cavities were filled with a thin sanguinous exudate; enteritis was well established and inflammatory changes were present in both the lungs. The umbilicus was normal. On cultural examination of the exudate a *Diplococcus* infection was detected, indicating that the infection was an intra uterine one.

Stafseth *et al.* (1937) reported similar findings and cultures revealed the presence of haemolytic streptococci. They believed this as a cause of repeated losses of litters by the same

bitch and stated that the puppies derived the infection from adult carriers. Stafseth (1940) later failed to reproduce the disease with streptococci in pure or mixed cultures and suggested that viruses or other pathogenic factors might either be provoking or predisposing factors.

Hare and Fry (1938) recorded  $\beta$ -haemolytic streptococci as infection in the suckling puppies, was isolated from the pharynx and the vagina of bitches in kennels in which disease was prevailing. From the puppies, they isolated streptococci, sometimes mixed with *B. coli* and staphylococci.

Stableforth (1938) reported that majority of the organisms recovered from the affected dogs were of Lansfield group G. He stated that streptococci were associated with many conditions causing serious losses amongst dogs.

Minnet and Elis (1940) expressed doubts with regard to the pathogenicity of haemolytic streptococci since these organisms were also recovered from a number of normal breeding bitches.

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# Chapter 28

## Herd Infertility

In the economics of livestock Industry great importance is attached to the maintenance of high fertility in the herds. By a rigid sexual health control it is possible to detect the subfertile, infertile or sterile animals in good time. On certain occasions, only a very few animals may be found infertile and the causative factors may also differ to a great extent. But when a large number of animals are detected to be infertile, it becomes a herd problem. By recognising the concept of herd infertility one has to make necessary investigations to tackle the problem.

In the Dairy Industry considering the problem of land, feeds, labour, transport and services of outstanding bulls, it is always economical to have a reasonable number of animals in a herd limited to the land in position so as to meet the home grown supplies of fodder at a very low cost. It is seen in general that the greatest amount of infertility in cattle and buffaloes is of herd significance and is of much higher economic importance than sporadic cases of infertility.

The potential breeding efficiency of male and female must be considered in their relationship to the unit or the herd. The problem of herd infertility

usually crops up only in the presence of one or more adverse factors to which the animals are predisposed resulting in lowered breeding efficiency. It is also true that when close in-breeding is done there is inherent danger of the possible presence of hereditary defects which might interfere with the fertility as a whole in the herd. Sporadic female infertility of a non-transmissible type can never become of herd significance, but sporadic male infertility invariably results in herd problem.

### Conception rate of the herd

A satisfactory conception rate in a herd can only be maintained with the high degree of breeding fitness in majority of animals in the herd. It is usually observed that beef and draught type breeds claim better conception rates than the dairy type.

Breeding health can be judged from the conception rate within the herd. High conception rates within the herd can be maintained to a certain extent by making adequate provisions of timely services from high fertile bulls over a suitably long period of time to give enough chance to the cows to express their fertility at proper time. The conception rate of a herd should be

estimated on the percentage of females becoming pregnant to the first service, percentage holding to the second service and so on. The conception ability of the individual animals and of the herd will thus be known and it will not be vitiated by the presence of individuals having sporadic types of transient forms of infertility. It is possible to ascertain the accuracy of the conception rate by subjecting all females to pregnancy diagnosis within 6 to 8 weeks after service.

In the assessment of herd fertility, one should not rely only on conception rates, as this method of measure does not account for failures during gestational period, birth of a dead foetus, weak or oversized calves, monstrosities or for post parturient genital abnormalities. If the foetus is dead during gestational period, the fertility of the female based on conception rate only is not altered but it does lower the calving rate. In all considerations calving rate is the most important indication of the overall breeding efficiency in the herd.

#### **Male and Female relationship in herd infertility**

The breeding efficiency of a herd is mainly dependent on the fertility levels of the bulls and cows. Fertility can be considered as a summation of the interplay of the reproductive functions within the individuals of each sex. The breeding fitness of one cow may differ from another one in contact in the same herd. Similarly the breeding fitness of the bull may also alter from day to day depending upon the quality of semen and the frequency of services. It is likely that due to adverse intra-uterine conditions, the spermatozoa may become weakened or even destroyed. A very high fertile bull may main-

tain the overall breeding efficiency of females including those suffering from slight degrees of genital malfunctions due to infective or non-infective factors. Whereas, if a bull of low fertility is used it invariably results in overall low breeding efficiency of the herd. It is also the common experience that a bull of slightly low fertility may pass unnoticed when he is used to serve very highly fertile cows. The significance of the male to female relationship within the herd is always increased in the presence of venereally transmissible diseases. Individual animals may vary in their resistance to coital diseases. The bull may act as a greater source for transmission of infections to a large number of females to which he is mated and at times the infection can be transplacental. In the male, the lowered fertility may result either in a temporary or permanent breeding inefficiency. In general, the cow shows a greater tendency and susceptibility to become easily involved in the lowered fertility group. But individual influence is not reflected on the herd as a whole except transmission of coital infections indirectly through the services of a bull.

Infertility in a herd is usually associated with the presence of number of adverse factors which interfere with the normal breeding efficiency (Fig. 92). Certain factors are apparent while-as certain factors are hidden and overall inefficiency is the net result of combination of both.

#### **Influence of various factors**

The inherent fitness of animals to produce is largely predetermined by the hereditary influences, however, this efficiency in turn is deeply influenced by the environmental factors. It is commonly observed that particular environ-



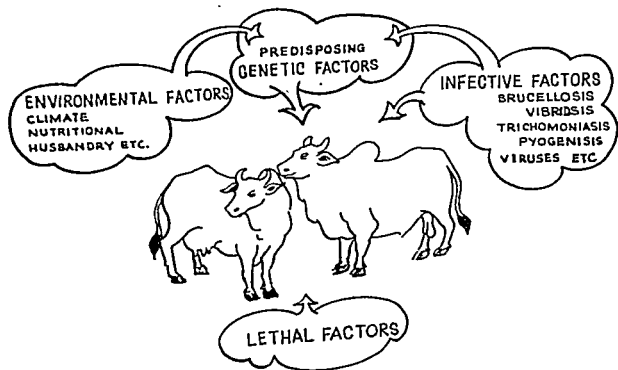


Fig. 92. Factors associated with bovine infertility.

mental conditions suitable for a herd may not be conducive to others. Whatever may be the inherent potential, it is always advisable to avoid adverse environmental influences wherever it is possible. Conditions of overstocking, lack of spacious accommodation and poor hygiene predispose the stock to known and unknown infections.

### Diagnosis.

In the assessment of herd infertility a detailed history should be obtained from the owner, which should include (1) size of the herd, (2) breed, (3) the managerial practices, (4) feeding standards, (5) data of all calvings, service dates and (6) reproductive disorders — abortions, retained afterbirth, abnormal discharges and infertility treatments. Cognisance should also be taken of all the fresh introductions to the herd.

The clinician should make a comprehensive study and then examine each

cow irrespective of the symptoms of breeding inefficiency. Each examination should be made in relation to the bull. If bulls are maintained in a herd for natural service, they should be critically examined. In certain types of herd infertility it can apparently be seen that the bull is actively involved.

During the recent years, large number of animals are being bred by artificial insemination to accrue the benefits of superior germ plasm. In such instances, difficulties have been experienced in the assessment of herd infertility since the bull's abilities to fertilize are not clearly known and the infertility investigations are mainly focussed on the females without reference to male relationship. Quarterly reports of bull's conception rates may become available but it takes pretty long time to obtain his calving rates. In a number of instances, the cows apparently appear normal but require repeated inseminations to settle. Under such conditions confusion always occurs if the cows in question need

any treatment or the semen of the bull has low fertilizing capacity. Though Artificial Insemination has practically eliminated the fear of transmission of coital infections, one should not ignore the likely possibility of transmission of other types of infections through semen when large number of cows may get involved. The presence of mixed infections is also a likely factor. Veterinarian should therefore carry out investigations without any bias.

### Prognosis

The chances of recovery are very variable depending on the causative factors and prompt attention in respect of the infective conditions. If breeding inefficiency is due to the hereditary weakness, no treatment will be of any use to save the breeding lines. One should only try to safeguard the herd from the sporadic instances of the male infertility.

### Control

For the maintenance of high breeding efficiency, it is primarily essential to maintain good level of general health and thus prevent the onset of debilitating diseases. Animals do resist adverse influences which might interfere with the genital functions, but the resistance is largely dependant on the inherent power of constitutional fitness, to a great extent. It is also greatly influenced by the extrinsic factors experienced by the individuals during the growth period and in adult life.

Control of hereditary transmission of anatomical and functional abnormalities is relatively easy. However, there are possible endogenous hereditary tendencies associated with a lowered resistance to various environmental

conditions and to infective factors, which are difficult to identify and control. It is difficult to diagnose the endogenous factors and as such it is better to aim at the preservation of fertility rate than to devise various remedial measures to restore fertility.

In the control measures, disease free intra uterine life of the foetus should also be taken into account. Growth is entirely dependant upon how an individual gets an opportunity to develop in a disease free womb.

The various factors vary in their impact on maintenance of fertility or predisposing them to subfertility and infertility in different age groups. For example, certain products from the mother, give protection to foetus during intra uterine life and others like colostrum ensure protection after birth. During growth, alterations do occur in the genital organs and their functions and depending on the food habits, variations also occur in their resistance which in turn influence the chances of exposure to genital disease.

Dairy cows and buffaloes have to undergo a very heavy strain due to the effect of gestation, lactation and the stress caused by the utilization of vast amount of food materials and its conversion. This should be given due attention while considering the problem of herd infertility. It is necessary to give adequate opportunity to rebuild strength and resistance. In a self contained herd, the risk of exposure to genital diseases due to infective factors is practically nil. Trouble usually sets in with new introductions or transport of cows from the herd either to markets or shows.

A very great care is necessary while introducing a new bull in the herd. It

is unwise to allow services of a bull for outside cows so also it is risky to borrow one from other herds.

Control measures are largely dependant on the elimination of the causative factors. The method of breeding by artificial insemination has certainly helped in reducing the problems of herd infertility. Maintenance of upto date records is of great help, in adopting effective control measures. In majority of instances the herd infertility is either due to the wide prevalence of Brucellosis which is particularly marked under Indian conditions or due to Vibriosis or Trichomoniasis as is

commonly observed under continental conditions. The control measures will differ in each case and to break the chain of infection, it is highly advisable to use semen from disease free bulls. There are several instances on record when herds have been wiped out due to gross inefficiency of breeding. In such instances feeding animals purely for maintenance purposes only becomes uneconomical. In the normal course, therefore the control measures, should be focussed to give necessary protection to animals in self contained herds. Maintenance of animal health in optimal condition will ensure higher breeding efficiency and production.

# Chapter 29

## Sexual Health Control

In the management of a herd, sexual health control gives an effective check on the reproductive performance of the male and the female. All possible observations concerning health and reproduction have to be systematically recorded from time to time. In this connection, the proforma of the Life History Card of the Female is very ideal to record all possible information from birth onwards.

The age at which a heifer attains puberty is important, since early sexual maturity and conception is conducive to optimum production. It is not always possible for a veterinarian to observe various stages personally and as such it is essential to make the farmers acquainted with certain aspects such as diagnosis of heat, type of normal oestrus discharge, discharge in pathological conditions, symptoms of mastitis and metritis, signs of parturition, time required for expulsion of placenta and whether the placenta and lochial discharge is normal or otherwise.

It will be seen from the proforma that the following information should be recorded for a correct assessment.

Age of the heifer at first heat.

When recording heat, it is necessary to mention, if it was intense, medium,

weak or prolonged; the date of service and the name of the bull, whether served naturally or by A.I. If by A.I., the quantity, quality and age of the semen should be recorded. If the animal repeats to service, then the date on which heat is expressed and the type of discharge, normal or abnormal, should be recorded. In case she does not repeat to service a veterinarian should examine her six weeks after the last service to carry out pregnancy diagnosis. If found pregnant she should be re examined at 3 month's stage to confirm the earlier diagnosis. In case the cow repeats to service and the discharge is not normal her genitalia should be examined per rectum and diagnosis and treatment recorded on the life history sheet.

A note on parturition should be maintained. Date of calving should be mentioned and the period of gestation calculated from the last date of service. Similarly, dry period for previous calvings should be recorded. Size of the litter, sex of the offspring and particulars of placenta should be recorded. At the end of each lactation, the total milk yield and number of days in milk and the service period should be entered.

Information whether the animal has been tested against T.B., J.D. and

Brucellosis need be mentioned. Preventive vaccinations done against H.S., B.Q., R.P. & F.M.D. should be recorded. Similarly, mention should be made if calfhood vaccination was done. If the animal has suffered from any ailment this should be recorded on the card in the Remarks Column. Details regarding the age at maturity; age at first calving; average of interval between calving and first observed heat; intensity of oestrus; period of gestation; intercalving period; time required for expulsion of placenta; number of services per pregnancy; period of reproductive and productive life should be calculated and entered. Reasons for disposal should be noted. A note also should be kept if there is dystokia, abortion, still birth and retained after-births so also the occurrence of malformations in the dam or her progeny; clinical findings on genitalia, venereal and general diseases. Treatment if any, should be properly recorded. In case any material has been sent to the laboratory for investigations, results thereof should be mentioned. Post-mortem findings if any, should also be recorded.

Veterinarian visiting the Farm should first study the records. Before any investigations or examinations are undertaken records and history will give a good idea about the reproductive health of the stock. Thereafter, he should take into account seasonal and managerial conditions and also the feeding standards. At the time of his visits, it is advisable that he should have a good look at the entire herd, young stock and heifers attaining puberty, adult females and breeding bulls and make a note on the general conditions. From the scrutiny of records he should make a note of the recent calvers, ones which have been served 5-6 weeks

before, those which are repeating to service, females which have not come in heat even after 5-6 months after calving or having evidence of prolonged lactational dioestrus, those voiding abnormal type of discharge, and the ones which are reported to have perceptibly gone down in their production within few months after parturition. Females which have a history of dystokia or retained afterbirths in the last calving or ones with history of abortion and any other conditions need attention. History cards of such animals should be properly scrutinized and examinations made thereafter. Those which are due for pregnancy diagnosis and those in anoestrous conditions should be examined first, whereas, others with the history of abortion should be examined at the end.

As far as possible, the animal should be examined in its byre or shed in standing position with very little restraint so as to avoid all possible excitement. This will give greater ease for per rectum examination. In this manner it is possible to work at ease avoiding any possible loss of time. Ferocious animals, however, should be secured in the crate to avoid chances of overriding and slipping. Material from suspected cases such as abnormal mucus discharge from vagina and uterus and cervical swabs from repeat breeders should be collected and subjected to bacteriological examinations, along with blood sera. Timely treatment will thus be possible.

Cases in which day to day treatment has been prescribed for vaginitis, cervicitis, metritis and chronic endometritis, the local veterinarian should be requested to follow up the treatment and these cases re-examined at the next visit.

Service record of the bulls should be properly studied with particular reference to their conception rate. New bulls should be introduced in the herd after proper andrological examination. In case of natural breeding the female should be watched for genital discharges prior to and following each service. In the examination of a bull close observation should be done with regard to the size, consistency and placement of the testicles. Behaviour of the bull during natural service should be watched to detect deviations.

As a rule whenever there are epidemics breeding operations should be stopped. Foot and mouth disease virus affects the bull testis. Bulls with mild FMD infection require 3-4 months to resume normal spermatogenesis and regain their normal breeding efficiency (Sharma 1969).

If rigid sexual health control is to be exercised the veterinarian must visit the farm as frequently as possible. Regular check up under the sexual health control service will be of immense use in enhancing the fertility rate and it also helps to weed out the sterile ones. Culling of the unproductive and sterile ones in good time proves beneficial.

In spite of the cost involved on the technical aid in instituting sexual health control service it is experienced in general that such an aid always proves beneficial.

## REFERENCE

- Sharma N C (1969) Impact of Foot and Mouth Disease on Sexual behaviour and semen quality in cow and buffalo bulls including study on testicular tissue. M V Sc Dissertation. Maharashtra Agr. Univ. Bombay.

# Chapter 30

## Udder Health Control

During the period between birth and sexual maturity the mammary glands develop an extensively complicated duct system together with a good amount of adipose tissue and progressive growth of mammary glands occur during pregnancy (or pseudopregnancy in bitches) when the new elaborate duct system acquires terminal lobules which consists of subdivision called alveoli.

The development of the mammary glands is initiated at sexual maturity and is under the influence of ovarian hormones. Subsequently it is influenced by the altering proportions of these hormones during different phases of reproduction viz. oestrus, pregnancy, parturition, involution of uterus, lactation and lactational dioestrus period. It is usually experienced that whenever the animal becomes a problem breeder or suffers with some reproductive disorders, the milk production is always affected. The reduced production may be related to certain changes in the udder. It is therefore necessary to take care of the predisposing factors during the prolonged period of reproductive failures which may lead to suppression of mammary activities. The role of other hormones like prolactin is not fully known.

### Effects of reproductive hormones on udder function

Oestrogens are believed to initiate udder function and parturition. High levels of oestrogens inhibit milk secretion. Stimulus during milking maintains secretion of several important pituitary hormones at high levels which is necessary for normal udder function. Before parturition this neurohumoral mechanism is not present to initiate lactation. Both for initiation and maintenance of lactation prolactin is essential. S.T.H. exerts greater ability to increase established milk yields in cattle than other hormones.

Administration of oestrogenic compounds during lactating phase either for inducing oestrus or as a part treatment for sub-fertility or infertility may inhibit or suppress milk production. Larger doses of oestrogens inhibit lactation by decreasing thyroid function, depressing appetite, increasing adrenal cortical function, increasing nervousness and such other factors. It is common observation that milk yield is reduced or suppressed in animals exhibiting intense oestrus. During gestation prolactin secretion remains too low to induce abundant lactation because of the predominance of progesterone over oestrogens. At term

there is a marked decline in both oestrogen and progesterone leaving the mammary gland to prolactin stimulation. At the same time sufficient oestrogen is still present to increase pituitary prolactin secretion and to initiate lactation (Meites, 1959).

In most of the countries a number of abortions in cows and buffaloes are annually reported to occur due to brucellosis. Abortions usually occur during the latter part of gestation and certain number of animals do start lactating. The milk yield is little and due to ignorance of the farmer such milk is usually marketed for human consumption. Under Indian conditions and in other tropical countries milk is usually heated before use but the dangers of transmission of *Brucella* infection cannot be over ruled. It is advisable to dry-off all such aborted animals.

Retention of placenta usually occurs in abortions due to *Brucella* or any other infection resulting in metritis. The symptoms will vary depending upon the intensity and in severe cases when there is pyrexia it will lead to suppression of udder function.

An acute septicaemia may develop within 24 to 48 hours after calving from uterine or mammary infections. In severe cases lactation is greatly suppressed.

Tubercular lesions in the udder may be found in the generalised tuberculosis.

Bacterial infections such as streptococcus uberis, *S. agalactia*, *Staphylococcus aureus*, *Corynebacterium pyogenes*, *Pseudomonas aeruginosa*, *B. coli*, *Actinobacillus*, *Mycobacterium tuberculosis* may cause mastitis. The flare up is very rapid and as such prompt treatment with antibiotics is advocated. The

severity of infection is variable and response to treatment is in accordance with the diagnosis and prompt measures. Emptying the udder at least thrice a day is always beneficial in reducing the tension from the mammary glands.

Infective conditions of the uterus always reflect on the udder. Lowered production on successive days of parturition is an indication of infective condition of the uterus. In such a condition, the uterus has to be checked and treated if necessary with a course of antibiotics.

### Methods of milking

There are two methods of milking viz. (i) by hand and (ii) by machine.

#### HAND METHOD

Milkers have to be trained for free hand milking. On no account undue pressure by thumb should be applied. Depending upon the type of teats, the hand pressure has to be applied to the teats while milking. Undue pressure and pulling is likely to cause sore condition of the teats as well as may set up inflammation of the teat canal. Any wounds abrasions or bruises to the teats particularly at the sphincter should be treated promptly and fly repellent applied to ward off flies. Injuries particularly at the base of teat start extending since in milking animals the teats have to be pulled while milking. Deep wounds causing sinus or fistula are difficult to treat and may lead to mastitis. Prompt surgical measures may prove beneficial.

#### MACHINE MILKING

In the machine milking method preparation of udder and teats prior to milking by chlorinated wash and immediately drying before the application of



teat cups is most essential. The suction cups have to be properly sterilised before use. Milkers have to be trained to keep a constant watch after the cups are fixed to note the pulsation and the amount of milk drawn. Undue suction of the teats after the milk is drawn may lead to capillary bleeding with consequent inflammation of the udder and

teats. Little hand stripping is necessary after removal of suction cups.

# REFERENCE

- Meites, J. (1959). Mammary Growth and Lactation. In: Reproduction in Domestic Animals, Ed. Cole, H. H. & Cupps, P. T. Vol. 1. Academic Press, N.Y.

PART III

**ANDROLOGY**

# Chapter 31

## Reproductive Organs in the Male

In all domestic species the organs of reproduction are more or less similar in pattern. The reproductive system of the male can be divided in three parts viz.

- I. Primary sex organs — Gonads or Testicles including scrotum.
- II. Accessory sexual glands and ducts — Epididymis, Vas deferens, Vesicular glands (seminal vesicles), Prostate and Bulbo-urethral glands.
- III. External genitalia or organs of copulation — Penis.

### Embryological development of the reproductive organs

The urinary and genital systems are closely associated as far as embryonal development is concerned. Both these sets arise in intermediate mesoderm or mesomere of somites. The ducts open into urogenital sinus and discharge their contents through the urogenital orifices. The actual openings are lined with ectoderm.

As regards the origin of germ cells Nalbandov (1958) mentions "that germ cells originate extragonadally in the yolk sac endoderm and that they migrate from there by amoeboid movements (mammals) or via the circulatory

system (chick), to the genital ridge where the first microscopically demonstrable aggregation of germ cells occur. Embryological analysis of several species shows quite clearly that the formation of a gonad depends on the arrival of germ cells and that the genital ridge alone is incapable of developing into anything resembling a gonad."

Primordial germ cells get located on the ventromedial side of the mesonephros where they multiply and the resultant thickening is termed as genital ridge. The undifferentiated gonads of the early embryonic life differentiate in the male into testis and in the female into ovaries. In almost all species of farm animals, the testicles descend from the abdomen and are located permanently in the scrotum. It is from the temporary kidney of the embryo (in the mesonephros) 12-15 tubules remain functional and the rest disappear. These tubules form the efferent ducts of testes and establish connection between testis and excretory canal i.e. the Wolffian duct. Epididymis, ductus deferens with its ampulla and seminal vesicles develop from the Wolffian duct and open into the urogenital sinus.

A very small portion of the Mullerian duct persists in the male from which

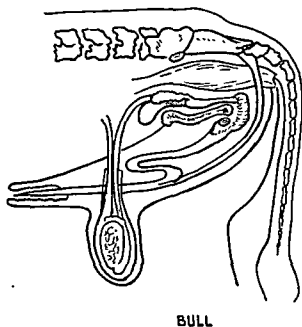


Fig. 93. Reproductive organs of the bull. rudimentary uterus masculinus covered by prostate or pea sized mullerian cysts may be found in the urogenital fold of some bulls (Blom and Christensen, 1958). The spermatogonia arrange into irregular cords of cells which acquire lumen and connect with tubules growing in from mesonephros. The testicular portions thus form the seminiferous tubules and nephric portion forms the efferent ducts.

### Reproductive organs of the cow-bull (Fig. 93)

#### SCROTUM

It is a cutaneous pouch in which the testicles are located. In the bull, the scrotum is elongated and in-tact. The hair on the scrotum are very scanty. Tunica dartos is found under the scrotal skin. This is composed of smooth muscle fibres with white fibrous and elastic connective tissues. This coat of tunica dartos surrounds both the testes and forms a partition between the two halves of scrotum called as 'septum scroti'. The next layer tunica vaginalis communis which is of white dense

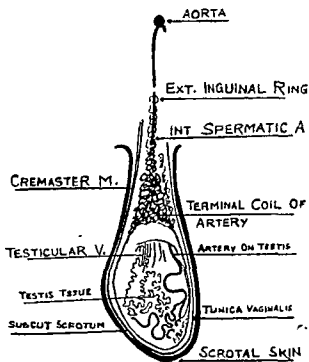


Fig. 94. Scrotum of bull showing different structures.

scrotal fascia surrounds the two halves of scrotum separately. Centrally, it is covered by the processus vaginalis which is an evagination of parietal layer of peritoneum (Fig. 94).

Raghavan (1962) reported the length of scrotum in Indian bulls as about 22.5 to 25.0 cm. during non-contracted condition.

**Functions** - The chief function of the scrotum is to hold the testes external to body and to provide thermo-regulatory mechanism in most of the mammals so as to enable the testes for the optimum production of sperm at a temperature lower than body temperature by about  $2^{\circ}\text{C}$  —  $7^{\circ}\text{C}$ . This is accomplished through the two external cremaster muscles and dartos which draw the testes close to abdomen during cold weather and let down the testes below for cooling when the environmental temperature is high. Exposure to cold causes tunica dartos to contract and

scrotal skin to shrivel, shortening the size of scrotum and the testes are pressed upwards close to abdomen.

Hot weather may lead to the breakdown of thermoregulatory mechanism leading to degenerative changes in germinal epithelium. Complete sterility is observed in bilateral cryptorchids, since the testicles are held high up in inguinal canal at body temperature. However, it is a common experience that in cryptorchid conditions, the sexual vigor is not affected i.e. the production of testosterone by the testes is not affected at higher temperatures.

### The Testes

The testes are the primary sex organs with two fold function of producing the sperm and secretion of male sex hormone. The testicles in the bull are

elongated, ovoid and they are placed with their long axis vertical in the scrotum. In the mature bull, they are 12 to 16 cm. in length and 6 to 8 cm. in diameter. The approximate weight including the epididymis may vary from 300 to 500 gm. depending on the breed, age and size of the bull. The testes are of unequal size and the left one is usually bigger. Raghavan (1962) stated that in Indian bulls the length, breadth (width) and thickness of testicle is about 12.5 cm., 6 to 7.5 cm., and 6.0 cm. respectively and it weighs 280 — 336 gm. Sane *et al.* (1966) noted the various dimensions of cow bull testicles as presented in Table 33.

Zanwar (1974) on a study of abattoir material of 13 pairs of testicles of cow bulls, reported the dimensions as given in table 34.

Table 33  
DIMENSIONS OF COW-BULL TESTICLES

Breed	Right Testicle			Left Testicle		
	Length (cm.)	Breadth (cm.)	Width (cm.)	Length (cm.)	Breadth (cm.)	Width (cm.)
Khillar	14.65	7.00	6.57	15.60	8.00	7.90
Nimar	12.50	7.45	5.45	12.55	8.52	5.60
Sindhi	13.10	10.20	6.00	12.90	9.00	5.90
Deoni	14.30	8.10	7.30	16.85	8.00	7.21

Table 34  
DIMENSIONS OF COW-BULL TESTICLES

Testes (With Epididymis)	(Right) Average	(Left) Average
Length (cm.)	12.071 ± 0.6585	13.333 ± 0.5109
Width (cm.)	3.861 ± 0.2680	4.195 ± 0.1243
Thickness (cm.)	4.317 ± 0.2141	4.60 ± 0.0594
Weight (g)	131.333 ± 19.1258	129.333 ± 19.6333

In the normal course the testicles in the bull are of equal size and firm in consistency. Testes move freely up and down in the scrotum. When the serous coats of testes, the tunica vaginalis propria and the tunica albuginea are cut, the yellow parenchyma becomes visible. The cut surface in median sagittal section shows a cord of connective tissue. The mediastinum testes which extends from the proximal pole to the distal pole measures 0.5 to 1.0 cm. in width. From this cord small streaks of connective tissue i.e. the septula testes extend between the seminiferous tubules due to which the testicular tissue appears slightly lobulated. In the cross section of the testes, it will be observed that the mediastinum appears as a central stellate figure enclosing the rete testis. These serve as collecting tubes for the seminiferous tubules.

#### HISTOLOGY

The septula testis extends from the mediastinum to the tunica albuginea. This divides the parenchyma of the testis into cone shaped lobules with their apex in the centre and base at the surface. These lobules which are the functional units are only partly separated in the normal bull testes, owing to the septula being thin and incomplete. The glandular parenchyma in each lobule consist of one or more convoluted seminiferous tubules. The tubules have a diameter of 0.1 to 0.3 mm. and are 50 to 100 cm. in length. The tubules sometimes anastomose with each other. The total length of the tubules in a pair of testis of the bull is as high as 5,000 metres.

Each seminiferous tubule consists of a basement membrane and a multilayered sperm producing epithelium. This

epithelium consists of two types of cells viz. (i) Germ cells i.e. spermatogonia, spermatocytes and spermatids in various stages of development differing in morphology and arranged in concentric layers and (ii) Sertoli cells also called as nurse cells or sperm mother cells or sustentacular cells which are slender pillar like structures perpendicular to the basement membrane.

The seminiferous tubules extend into the mediastinum testis and unite to form a network of ducts termed as the rete-testis. This is lined with cubical epithelium. Twelve to fifteen tubules arising from the rete testis further connect with the epididymis. These tubules serve as efferent ducts of testis.

The blood vessels, nerves, lymph vessels and the Leydig cells are contained in the connective tissue in between the seminiferous tubules. The Leydig cells are isolated groups of polygonal cells with large spherical nuclei and which are the chief source of the male hormone testosterone.

#### Functions

Prolific activity is the most distinguishing feature of testicular tissue. Willett and Ohms (1957) mentioned that one gram of testicular tissue in the bull produces on an average about 6000 spermatozoa per minute. In farm animals although spermatogenesis is a continuous process, great variations are usually seen with regard to testicular function from season to season and the plane of nutrition.

The testicular fluid produced in the seminiferous tubules carry the newly formed non motile spermatozoa through the rete testis. The tense consistency of the testicles is purely due to this fluid which is secreted under great pressure.

In addition to this, male sex hormone is produced by the bull testis under the influence of gonadotropins liberated by the anterior pituitary. The interstitial cells — Leydig cells secrete testosterone which is responsible for manifestation of secondary sexual characteristics.

### Epididymis

This is an elongated structure which is very closely adherent to the testis. It consists of head (Caput), body (Corpus) and tail (Cauda). The caput forms a flattened cup-shaped protrusion which is located at the proximal pole of the testis. It is U shaped, varies in outline and size practically covering 1/3rd of the front of the testicle. The caput is continued into a slender corpus running distally along the posterior border of the testis. At the distal pole of testis, the body enlarges into a well defined tail termed the cauda epididymis. In the adult bull, size of the cauda is conspicuous and is distinctly palpable. In the bull the entire epididymis consists of a single tubule, which is thrown into convolutions and measures 40 metres or so. The epididymis is more firm in consistency than the testis.

### HISTOLOGY

The duct of epididymis in the region of caput consists of two types of epithelial cells attached to the basement membrane, viz. secretory cells and ciliated cells having kino-cilia beating outwards. The detached ciliated cells commonly found in the semen are termed medusa formations and according to Blom (1944) they may be found in the ejaculated semen at the rate of one per 10,000 spermatozoa. The ductus epididymis starting from the centre of caput can be differentiated histologically and cytochemically into six distinct regions

(Nicander, 1958; Crabo, 1965). The basement membrane in this region is surrounded by circular layer of smooth muscle fibres increasing in thickness towards the cauda. Throughout the duct the lining epithelium consists of cylindrical cells with non-motile cilia (stero-cilia). The height of epithelium gradually decreases towards the cauda from 140  $\mu$  to 60  $\mu$ . The lumen of the duct measures about 1 mm. in diameter.

### Functions

Four major functions, namely transport, concentration, maturation and storage of the sperm are attributable to the epididymis.

#### (i) TRANSPORT

The transport of spermatozoa from the rete-testis to the efferent ducts occurs under the pressure of testicular fluid. Their further passage through the efferent ducts is aided by the ciliated epithelium and the peristaltic movements of the muscle fibres. Knudsen (1954) estimated a period of 7 to 9 days for the transport of sperm upto cauda.

#### (ii) CONCENTRATION

During the onward passage of the diluted sperm suspension, the epithelial cells in the region of caput absorb water, leaving a highly concentrated sperm cell suspension in the cauda.

#### (iii) MATURATION

The maturation of sperm occurs in the epididymis due to the secretions from epithelial cells. During the maturation process, the proximal protoplasmic droplet migrates to the distal end of midpiece (distal cytoplasmic droplet) in the bull. Such a migration occurs in the caput and hence sperms with

distal cytoplasmic droplets are usually found in the cauda.

#### (iv) STORAGE

The cauda epididymis serves as a sperm storage depot. According to Bialy and Smith (1958), about 50% of the sperm are stored in this region where the epididymal duct is relatively wide. The sperm are in quiescent stage of metabolism which is due to the optimal intra-epididymal conditions. Even after ligation of the epididymis, sperm can remain viable upto 60 days and maintain ability to fertilize.

#### Vas deferens and the accessory sex glands

Vas deferens is a conspicuous duct, which extends from the tail of epididymis to the urethra. It has an outer diameter of about 2 mm. The ducts are firm and cord-like due to the thick muscular walls. The vas deferens is convoluted in form near the cauda and its course is parallel to the body of epididymis. The vas deferens is straight at the caput and along with the blood vessels, lymph vessels and nerves and cremaster muscle it forms the spermatic cord. It passes through the inguinal canal into the abdominal cavity. On entering into the abdomen, the vas deferens separates from the spermatic cord, passes backwards to open into the urethra. Before opening into the urethra, the terminal parts enlarge to form the ampulla (ampulla ductus deferentis). The thickening of the duct at the ampullar region is due to the presence of abundant glands in its wall. The two ampullae traverse under the body of prostate and open on colliculus seminalis by a slit like opening. The ampulla occupies a position either dorsal, ventral, intermediate or close to seminal

vesicles (Blom and Christensen, 1947; Kanagava, 1960). The ampullae serve as a store house for the spermatozoa.

The function of vas deferens is to transport sperm from cauda to urethra. This is due to peristaltic movement of the duct.

#### Vesicular glands (Seminal vesicles)

In the bull, the seminal vesicles are paired glands lobulated and situated in the urogenital fold lateral to ampullae above the neck of the urinary bladder. In an adult bull, they measure 10 cm.  $\times$  5 cm.  $\times$  2.5 cm. The size varies considerably and also variations are observed in lobulation from individual to individual. A yellowish glandular tissue is usually seen on the cut surface. The main excretory duct located in the centre of the gland extends backwards under the prostate to open in urethra together with the ductus deferens through two openings viz. ostia ejaculatoria. The position of ampullae and vesicular glands varies between individuals and are easily palpable per rectum.

#### HISTOLOGY

In the bull, vesicular gland is lobulated — each lobule being separated by strong muscular septa. Secretory tubules are branched tubular glands with a diameter of about 0.3 cm. They are lined centrally by large columnar cells about 25 to 30 microns long and peripherally by cells containing large droplets of lipid. The muscular layer consists of inner circular and outer longitudinal muscle fibres which in turn are surrounded by a fibrous coat.

#### Functions

Seminal vesicles have a glandular structure secreting faint, opalescent and



sticky fluid. The secretion contains proteins, potassium, citric acid, fructose and several other enzymes in high concentration (Mann *et al.*, 1956). In mammals most of the seminal fructose comes from the vesicular glands. On account of its high flavin content it has distinct yellow colour. In the bull, the secretion of vesicular glands constitutes about 50% of total ejaculate with a pH varying from 5.7 to 6.2.

### Prostate

In the bull, the prostate gland surrounds the urethra and is composed of two parts, the body of prostate (*pars propria*) and the *pars disseminata* (*cryptic prostate*).

The body is a small mass which stretches across the dorsal surface of the neck of bladder and the beginning of the urethra. It is about 1.25 cm. in length transversely and about the same in width and thickness. It is covered dorsally by fibres of urethral muscle.

*Pars disseminata* or the glandular part surrounds the pelvic urethra. It is thick on the dorsal part of urethra and thin ventrally.

### HISTOLOGY

Prostate is musculo-glandular and lobulated — each lobule being divided by trabeculae derived from outer fibrous coat. The trabeculae contain muscle fibres. Each lobule contains a central axial duct and this has the appearance of branched tubular gland. The ducts and tubules are lined by cubical or columnar epithelium.

The prostatic ducts open into the urethra by about 30 ducts in rows on the *colliculus seminalis*.

### Cowper's or Bulbo-urethral glands

In the bull, these are paired, round and compact glands with a dense capsule and are of the size of a walnut. They are located above the urethra near the ischial arch. One excretory duct is formed from the secretory ducts from each gland which has a length of about 2.3 cm. These ducts open in the mucus fold of urethra.

### HISTOLOGY

The parenchyma consists of tubules and acini. Muscular fibres are much less and no distinct lobulation exists. The acini are lined with columnar epithelium that yields mucus like secretion.

### Function

The secretions of the prostate and Cowper's gland are of apocrine type i.e. the central part of the cytoplasm of the cell is transformed into secretion and is shed off. In the bull dribblings from prepuce noticed before mounting are the secretions from the prostate and Cowper's gland. The secretion cleans the urethra of the deleterious effects of urine.

In incomplete ejaculations, secretions obtained from these glands are watery, clear and sperm free. The pH of both fluids varies from 7.5 to 8.2 (Blom, 1950).

### Urethra

Urethra in the male is a common excretory passage both for urine and semen. It has three distinct portions viz. (a) the pelvic part is about 15-20 cm. in length, cylindrical, enclosed by the urethral muscle and situated on the pelvic floor, (b) The bulb of urethra is the extra pelvic part situated at the

ischial arch and bends to the ventral aspect of the penis, (c) Penile part belonging to penis proper.

A hazel nut sized prominence located posterior to the neck of the bladder is termed *colliculus seminalis*. On the dorsal part of *colliculus seminalis* can be seen the orifices of the ampullae and excretory canals of the vesicular glands. The *colliculus* chiefly consists of cavernous tissue which closes the neck of urinary bladder during ejaculation and thus it prevents simultaneous entry of semen to the bladder.

### Penis

Penis is the copulatory organ which has a double function viz. (a) expulsion of urine and (b) deposition of semen in the vaginal tract of female. In the adult bull, it averages about 120 cm. in length and has a smaller diameter.

The penis consists of root, body and glans. The root is attached to the pelvis by two crurae of penis. The body of penis essentially consists of erectile tissue, *corpus cavernosum* which has the sinusoid like spaces that get filled with blood during erection resulting in stiffness of the fibro-elastic structure of penis without much enlargement of the organ. To a certain extent the erection of penis is due to temporary inability of blood to return from cavernous spaces of penis. A part of erectile tissue also surrounds urethra termed *corpus cavernosum urethrae*. Major part of body of the penis is arranged in the form of a sigmoid flexure which gets straightened during erection thereby increasing the length of penis by about three times.

The glans penis which is the terminal part of the organ is pointed and slightly twisted (Fig. 95). On the left side of the tip is a groove in which the

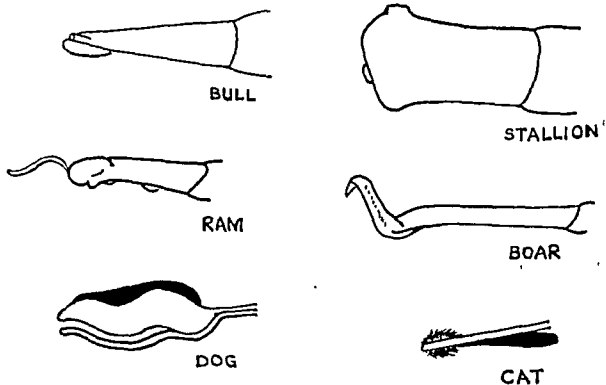


Fig. 95. Glans penis in domestic animals.

external urethral orifice is located. The two retractor penis muscles originate from each side above the anus, extend up to glans penis and serve to retract the penis back into the sheath after ejaculation or when sexual stimulus is suppressed. It also helps to keep the organ in fixed position during non-erectile state in the sheath.

### Prepuce

Bhardwaj and Calhoun (1961) studied the mode of formation of preputial cavity and stated that penis was not free in the preputial cavity at birth in most of the domestic animals. It exists in the form of a fold — balanopreputial fold splitting of which occurs under hormonal influences. The separation commences at 3-4 months of age and is complete by the time, the male reaches puberty (Ashdown 1962). Abdel Rouf (1960) reported in Swedish Red and White breed that the prepuce was adherent to penis at birth. Separation was first noticed at 4 weeks of age and then 2 mm. of penis was exposed. Separation was then gradual and completed by 32 weeks of age.

In the bull, the prepuce or sheath is long and narrow. It is an invagination of the skin which encloses free extremity of retracted penis. Preputial opening is surrounded by a tuft of long and tough hair. The opening of the sheath is located at a distance of about 5 cm. behind the umbilicus. It is large enough to admit a finger. The cavity of prepuce is 35-40 cm. in length and 3-4 cm. in diameter. The mucus membrane of sheath is folded and has a number of coiled tubular glands which produce a fatty secretion. This secretion gets mixed up with cast epithelial cells and bacteria which forms the semisolid

crusts known as smegma preputi emitting offensive odour.

### REPRODUCTIVE ORGANS OF THE BUFFALO-BULL

The reproductive organs of the buffalo-bull are similar to those of the cow-bull.

#### Scrotum

The scrotum of the buffalo is 20-25 cm. in length and has a distinct neck. In swamp buffaloes it is about 10 cm. in length and the neck is not distinct (MacGregor, 1941). Bhattacharya (1960) observed that in Murrah buffaloes testes are present in scrotum at birth. However, in certain number there will be no descent of the testes in the scrotum at birth.

#### Testis

Toke (1967) observed the testicle measurement in one week old buffalo calves — the length as 1.76 cm. (1.28-2.25 cm.), breadth as 0.67 cm. (0.48-0.94 cm.), thickness as 1.05 cm. (0.68-1.60 cm.) and weight as 0.883 gm. (0.368-1.351 gm.).

Naik (1965) reported the length and width of the testes at 3 weeks age of buffalo calf as 1.55 and 1.2 cm. respectively. He further studied the postnatal development of testes of Murrah buffalo calves aged 1 to 130 weeks and observed the length and weight of the testes as 1.5 to 8.85 cm. and 0.8 to 25.5 gm., respectively.

Toke (1967) observed that the average diameter of the seminiferous tubules of one week old buffalo calves was  $34.26 \mu$  ( $23.4$  to  $39.6 \mu$ ) and in two week old calves the diameter was  $35.53 \mu$  ( $23.05$  to  $39.6 \mu$ ). The testis

is about 7.5 to 8.5 cm in length and 3.5 to 5 cm in width (MacGregor, 1941). The average length and the thickness of the testis (including epididymis) in Pandharpuri buffalo-bulls is  $14.21 \times 6.11$  cm for the right and  $15.0 \times 5.87$  cm for the left (Sane *et al*, 1966). Joshi *et al* (1967) reported the length, breadth and circumference of the testes (without epididymis) as  $7.60 \times 1.30 \times 12.20$  cm for the right and  $7.87 \times 1.33 \times 12.29$  cm for the left respectively. The weight of the right and left testes was 74.86 gm and 79.06 gm respectively.

Maurya *et al* (1968a) studied 110 sets of genital organs from the slaughtered mature buffalo bulls. They found no significant differences in the length, width and thickness of the right and left testes (Table 35).

Zanwar (1974) on a study of abattoir material of 15 pairs of testicles of buffalo bulls observed the measurements as presented in Table 36.

In the buffalo bull the caliber of the *seminiferous tubule* is smaller even to that of the ram and goat (Mukherjee *et al*, 1961).

Table 35  
MEASUREMENTS OF TESTICLES OF BUFFALO-BULL

Testes (with Epididymis)	Right Average	Left Average
Length (cm)	$11.18 \pm 0.951$	$11.27 \pm 0.878$
Width (cm)	$4.85 \pm 0.177$	$4.82 \pm 0.446$
Thickness (cm)	$3.99 \pm 0.126$	$4.03 \pm 0.476$
Weight (g)	$96.38 \pm 18.617$	$99.31 \pm 13.085$

Table 36  
DIMENSIONS OF BUFFALO-BULL TESTICLES

Testes (with Epididymis)	Right Average	Left Average
Length (cm)	$11.66 \pm 1.30$	$11.53 \pm 0.82$
Width (cm)	$1.03 \pm 0.13$	$1.26 \pm 0.17$
Thickness (cm)	$1.50 \pm 0.50$	$1.29 \pm 0.28$
Weight (g)	$57.00 \pm 2.00$	$71.00 \pm 17.03$

Table 37

## DIMENSIONS OF BUFFALO-BULL EPIDIDYMIS

Description	Length (cm.)		Breadth (cm.)		Thickness (cm.)		Weight (gm.)	
	Left	Right	Left	Right	Left	Right	Left	Right
Caput ...	3.50	3.48	3.92	3.87	0.76	0.77		
Corpus ...	8.59	8.73	0.99	0.99	0.29	0.29		
Cauda ...	2.40	2.41	1.78	1.69	1.56	1.55	5.42	5.45

## Epididymis

Joshi *et al* (1967) reported the length, breadth and thickness of the caput, corpus and cauda of the epididymis in adult buffalo bulls as under (Table 37).

## Ductus deferens and ampullae

Ductus deferens is similar to that of cow-bull.

Joshi *et al* (1967) observed the location of the ampulla as 55% in dorsal, 10% in ventral and 35% in intermediate position to vesicular glands. The length and diameter of the ampullae was recorded as 13.80 and 0.61 cm. for the right and 14.05 and 0.62 cm. for the left respectively.

## Vesicular glands

Vesicular glands in the buffalo-bull are relatively small compared to those in the cow-bull. The various measurements of the vesicular glands in the adult buffalo-bulls are as shown in Table 38 (Joshi *et al*, 1967).

## Penis

In river buffaloes Joshi *et al* (1967) reported the length of penis as 83.51 cm. whereas in Swamp buffaloes, Euri-

Table 38

## DIMENSIONS OF VESICULAR GLANDS IN THE BUFFALO-BULL

	Length cm.	Breadth cm.	Thickness cm.	Weight gm.
Right	7.14	3.05	1.14	11.01
Left	7.02	3.18	1.11	10.87

quez yap and Seldera (1964) reported the length as 56.72 cm. in flaccid state and 84.15 cm. during erection with an average diameter of 2.95 cm. Sigmoid flexure measured 17.23 cm. with average diameter of 2.79 cm. and the root of penis measured 4.99 cm.

The penis hangs clear of the abdomen by 15-30 cm. being attached thereto by triangular fold of skin running backwards from the umbilicus. Penis is adherent to abdomen except for the last 2-3 cm. or so in Swamp buffaloes (MacGregor, 1941). Glans penis is soft, thin, flexible and tapering to a fine point. It is 9.32 cm. in length and 1.67 cm. in diameter. Galea glandis is hardly noticeable. Penis is cylindrical in cross-section and not flat dorsoventrally. During erection, glans penis hardens and vibrates rapidly simulating the appearance of processus urethrae of ram during mating.

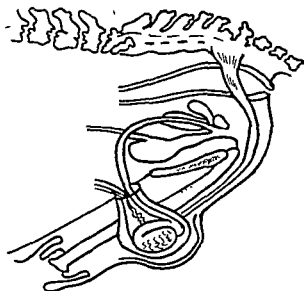
**STALLION**

Fig. 96 Reproductive organs of the Stallion

**Prepuce**

The prepuce is devoid of hair.

### REPRODUCTIVE ORGANS OF THE STALLION

In the stallion the scrotum is asymmetrical and less pendulous than in the bull. Testes lie horizontally in relaxed state but when cremaster muscles are retracted, they become almost vertical. They are ovoid in form measuring about 10 to 12 cm. in length, 5 to 8 cm in height and 5 cm. in breadth. The weight varies from 240 to 300 gm. (Fig. 96).

The ampullae are very well developed. Vesicular glands are 10 to 12 cm. in length, 5 cm. in width and 2.5 cm. in thickness. The pyriform sacs are smooth and elongated, their mucous membrane is thin and folded to form a network. The prostate consists of two lateral lobes and a connecting isthmus. Penis is cylindrical about 50 cm. in length in quiescent state of which 15 to 20 cm. is free in prepuce.

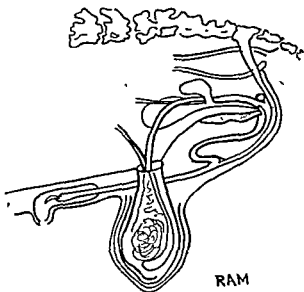
**RAM**

Fig. 97. Reproductive organs of the Ram.

During erection it increases to about 75 cm. in length. Glans is large. There is no sigmoid flexure. Prepuce has a secondary fold within the preputial cavity which forms the internal prepuce in which anterior part of penis is located. The glans penis is surrounded by a margin which is prominently denticulated and this mushroom-like formation is seen when the stallion withdraws penis after copulation. The urethral process protrudes as a 2.5 cm. long tube situated in a depression called as fossa glandis in front of the glans (Fig. 95).

### REPRODUCTIVE ORGANS OF THE RAM AND BUCK

The organs are similar to those in the bull except that the weight of testicle when compared in relation to the body weight is much higher. They are about 10 cm. in length and about 270-300 gm. in weight. Scrotum is shorter and has no distinct neck. In the buck at 180 days age the testicles are 1.80 cm. in length and 3.57 cm. in width. The axis of the testis is similar to that in the bull (Raja, 1977). In the buck, skin of the scrotum is covered with hairs. Left testis was

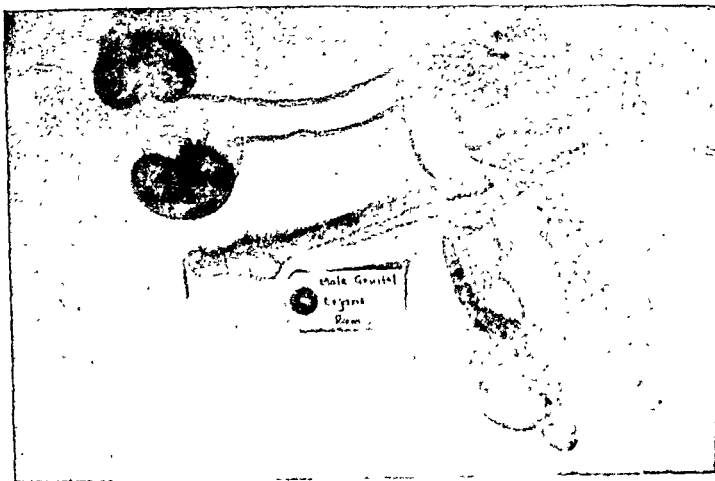


Fig. 97a Urogenital organs of Ram.

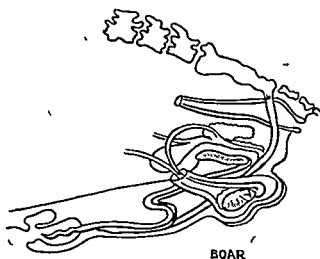


Fig. 98. Reproductive organs of the Boar.

found to be heavier than right. Similarly left epididymis was found heavier than right and sperms were observed in its lumen at 165 days of age (Harshan, 1977). It is covered with wool in Marino rams and few other breeds and in the rest, the wool is either scanty or devoid of it. The prostate consists only of pars disseminata. In the buck the bulbo urethral glands are relatively large. (Fig. 97).

The glans penis has a twisted, filiform appendage measuring about 3.4 cm. in length. This is known as processus urethrae — a terminal part of the urethra. During ejaculation, this filiform appendage rotates rapidly and sprays semen on os-uteri (Fig. 95).

In the buck penis is 30 cm. long with a well developed sigmoid flexure. The diameter of the penis is relatively small.

### REPRODUCTIVE ORGANS OF THE BOAR

The scrotum is situated just below the anus and is not well defined as in the bull. The testes are situated horizontally in the scrotum. The approximate size of the testicle is 10-15 cm. in length  $\times$  5 to 9 cm. in width.

The weight of the testicle varies from 500 to 800 gm. (Holst, 1949). The ampullae are comparatively small. The vesicular glands are very large, dense and measure  $12 \times 5 \times 4$  cm. and weigh about 180-240 gm. The Cowper's glands are cylindrical and lie along the pelvic part of the urethra and measure  $12 \times 4$  cm. (Fig. 98).

The penis is fibro-elastic organ and its terminal free part has a distinct cork-screw shape (Fig. 95). The sigmoid flexure is located anterior to the scrotum. The prepuce has a narrow orifice with stiff hair. In the boar the prepuce is about 9 cm. in length, 12 cm. in width and presents a preputial diverticulum, a blind cul-de-sac in its dorsal part. The pouch usually contains a mass of decomposed urine and mascerated epithelial cells which always emits a very characteristic and unpleasant odour. The smegma is responsible for the strong sex odour of boars which permeates their flesh and gives a disagreeable taste.

### REPRODUCTIVE ORGANS OF THE DOG

Scrotum is ovoid, globular, asymmetrical and pigmented. It is oily to touch and has a distinct scrotal raphae. It is located midway between the inguinal region and anus. The scrotal skin is sparsely covered with fine hair.

In the dog the testicular size and weight vary to a great extent from the large sized St. Bernard and Hounds to the small sized Pomeranians and Poodles. They are round or oval in form. The size varies from 2 to 4 cm. in length and 1.2 to 2.5 cm. in diameter. Weight of testis varies from 7 to 15 gm. Its long axis is oblique and is directed upwards and backwards. Rete testis is central in position. (Fig. 99).



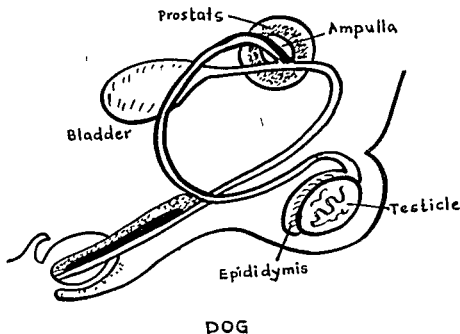


Fig. 99. Reproductive organs of the Dog.

The prostate gland of dog is relatively large, yellowish in colour, dense in structure and can be located at or near the anterior border of pubis when bladder is empty and contracted but when the bladder is full, it becomes largely prepubic. It is globular and it surrounds the junction of bladder and urethra. It has numerous ducts.

Prostate in the dog is subjected to great variations and is often found enlarged in older dogs.

Vesicular glands and Cowper's glands are absent in the dog.

The pelvic part of the urethra is very long (15-20 cm.) and enclosed in the prostate. This has clinical importance since enlargement of prostate may interfere with micturition. At the ischial arch, it has a well developed bulb due to the enlargement of corpus cavernosum urethrae. The urethral muscle is very strong.

The penis is provided with os penis and the glans extends over it (Fig. 95).

Anterior part of penis is called pars longa glandis and posterior part as bulbous glandis. It is due to the enlargement of the bulb that the interlocking of penis occurs during coitus which is peculiar to dogs.

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# Chapter 32

## Hormones of Reproduction in the Male

Reproduction can be defined as the process of multiplication of the individuals of a species. It is one of the most vital processes in the living beings, as the continuity of the species, depends on the production of its offsprings which grow, mature and again produce their progeny. This goes on from generation to generation. Thus, the process is called reproduction.

Apparently, the process of reproduction appears to be simple one but in bisexual animals, it is most complicated in the sense that both male and female are sexually incomplete as they are interdependent on each other for successful reproduction. Further, the reproduction involves the co ordination of many interdisciplinary systems, of which nervous and endocrine systems are most important. In addition to body systems, other factors such as nutritional status, climate, social set up etc. have also an impact on reproduction.

Hormone is a Greek word derived from the word "hermaio" meaning to arouse, to activate, to initiate, to stir up, to stimulate etc. and the word was first used by Bayliss and Starling in 1902. Hormones are physiological organic substances liberated by living cells of a restricted area of the organ, which diffuse

or transported to a site in the same organism, where these bring about changes that tend to integrate the component parts and actions of the organism.

### History

Claude Bernard employed the term 'internal secretion' for the secretions of specialised glands. Brown Sequard in 1899 accomplished self rejuvenation at the age of 72 by self injection of aqueous extracts of dog testes. The first definite isolation of a reproductive hormone was made by Allen and Doisy in 1923, with the discovery, that changes found in the vaginal smear of rats, during the heat could be produced in ovariectomised rats by injecting extract of liquid obtained from ovisacs of sows.

Hench *et al* (1949) announced that the hormone, cortizone from the adrenal cortex relieved clinical symptoms of rheumatoid arthritis. Simpson and Tait (1953) discovered the unidentified steroid present in the extract of the adrenal gland as aldosterone, this hormone regulates water and electrolyte metabolism. DuVigneaud *et al* (1953) determined the structure of oxytocin and vasopressin, polypeptide hormones, from the posterior pituitary. Discovery on structure, function and their synthe-

sis of many hormones has not come to an end:

### Hypothalamo-pituitary control

The control of reproductive phenomena is peculiarly intricate with an elaborate neuroendocrine circuit involving the pituitary gland, the gonads, the brain and the inter disciplinary interaction among other endocrine systems and influence the next organ in the circuit and thereby establish an optimal equilibrium. Hohlweg and Junkmann (1932) on the basis of cytologic evidence proposed the existence of a hypothalamic sex centre. The areas involved are the hypothalamus and the limbic system of the brain. Recent developments include neuro-humoral concepts of adeno-hypophysial control in which gonadotrophin releasing factors (GN-RH, FSH-RH and LH-RH) are elaborated from the median eminence and brought to adeno-hypophysis via the hypothalamo-hypophysial portal system, as direct innervation from hypothalamus to the adeno-hypophysis has not been demonstrated. The basal hypothalamus has cells or receptor elements directly sensitive to sex steroids, which form a mechanism for controlling and release of gonadotrophic hormones. Among its many connections, the hypothalamus interacts with a midbrain circuit including the reticular activating system (RAS) and with a limbic circuit. The interaction of hormones and brain functions co-ordinate pituitary gonad activity with appropriate sexual behaviour and other reproductive functions.

### Pituitary controls in bulls from birth to maturity

Macmillan and Hafs (1968) studied the pituitary and hypothalamic endocrine changes associated with reproduc-

tive development of Holstein bulls, from birth till one year of age. They found that the LH concentration was greatest at 1 month and then declined with increasing age, whereas pituitary content of LH increased irregularly to a maximum value at 6 months of age. However, it neither fluctuated abruptly with the onset or termination of puberty. The total plasma LH did not change significantly from birth to 2 months, increased at 4th month and increased again between 6th and 10th month of age. Although LH releasing factor could not be detected in hypothalami from bulls less than 5 months of age, the increase in plasma LH, between 6 and 10 months was associated with a marked simultaneous increase in levels of hypothalamic LH-RH. Concentration of pituitary FSH was greatest at 2 months but the content was greatest at 5 and 6 months and both declined after 6 months of age. The data suggest that puberty in Holstein bulls commences at 2 months and is qualitatively completed by 10 months of age. Greatest changes occur between 6 and 10 months of age.

### Sexual hormones in male animals

In males, the pituitary hormones FSH or SSH (spermatogenesis stimulating hormone) is responsible for spermatogenesis and LH or ICSH (interstitial cell stimulating hormone) stimulates the testis to secrete the male hormone "Testosterone". Testosterone causes the development of secondary sexual characters in the male and regulates the sex desire. It has been shown that androgens themselves have an influence on the process of spermatogenesis. Mann (1945) has shown that the principle sperm nutrient present in the seminal plasma is fructose. In bulls and buffalo

bulls the seminal vesicles furnish the major portion of seminal fructose. It has been demonstrated by Mann *et al* (1947, 1949) that the rate of fructose elaboration by accessory sex glands is directly related to the hormonal activity of testes. The initial fructose content in the semen therefore has been used as an indicator to know about the activity of Leydig's cells and indirectly the sex desire while the estimation of rate of fructolysis in semen helps to appraise the semen quality.

### Testicular function

#### (a) PUBERTY AND SEXUAL MATURITY

The process of growth in animals is for the most part under endocrine control and reproductive processes are primarily under endocrine control (MacDonald 1969).

Reproduction largely depends upon the co-ordinated function of the various endocrine glands. Emmens (1959) considered that in male relation between pituitary hormones (FSH and LH) and testis is not cyclical as with the ovary and is considered simpler. Puberty has been defined as the phase of bodily development during which the gonads secrete hormones in amounts sufficient to cause accelerated growth of the genital organs and the appearance of secondary sexual characters. It is believed that during immaturity, either the pituitary does not secrete sufficient amount of gonadotrophins or the testis is not fully sensitive to the action of gonadotrophins secreted by the pituitary. As soon as the threshold level of gonadotrophin is attained, Leydig cells start secreting enough androgens which bring about puberty by inciting secondary sexual characteristics and start of spermatogenesis. Complete maturity is an

advanced stage of puberty when all the reproductive organs attain peak functional activity and the individual enters a stage of full sexual vigour.

### Descent of the testis

The factors concerning the migration of testis into the scrotum are not thoroughly understood. However, one view is that the traction caused by gubernaculum testis by progressive shortening might be playing an important role. A probable contributory factor may be the increase in the intra abdominal pressure. Lastly hypophysis and the gonadal hormones have been shown to activate the descent of testis, as it has been shown that administration of gonadotrophic hormones cause descent of testis in human cryptorchids (Shapivo, 1930) and in monkey (Hamilton, 1938). According to MacDonald (1969) at birth the bull testes are in the scrotum. The Leydig cells are formed by 4 months. Androgen production rises slowly until 2 years of age then increases sharply until 5 to 7 years of age. Thereafter a slow decline occurs.

### The testis performs two functions

(i) Gametogenic and (ii) Endocrine androgenic activity

Androgens control the secondary sex characters of male and the secretion of seminal plasma. Even though spermiogenic and androgenic functions are complementary, at times these may not coincide. In the normal male the onset and duration of spermiogenic and androgenic functions do not differ frequently but under pathological conditions there is a selective derangement of one of these functions. Spermatogenesis and androgen production are either continuous

throughout the year, in year round breeders or throughout the season in seasonal breeders. The size of the scrotal sac is controlled by testosterone. The scrotum plays an important role in *thermo-regulation of testis*. The male sex hormone is secreted by Leydig cells. These interstitial cells are interspersed among the seminiferous tubules. Sertoli cells have also been shown to secrete small quantities of estrogens. The functions of testes are under the control of the pituitary gonadotrophins FSH and LH; FSH stimulates spermatogenesis and LH stimulates synthesis and release of testosterone. In the absence of gonadotrophins such as after hypophysectomy, the process of spermatogenesis ceases, the Leydig cells fail to secrete androgen and testicular atrophy occurs. For control of androgen release a feed back mechanism exists analogous to pituitary thyroid and pituitary adrenal system. Injections of large doses of testosterone will cause testicular atrophy, however, direct application of small amount of testosterone to the hypothalamus has been found to inhibit both spermatogenesis and the androgen release (Davidson and Sawyer, 1961). According to Vilar and Hertz (1958) genetic factors also play a role in the post pubertal activity of the testis. There are other sites of androgen production in the body. These are adrenal cortex, the ovary and probably placenta. Of them adrenal cortex produces most significant amount of androgens. Hill and Strong (1940) reported that under pathological conditions large amount of androgens also may be released from the ovary.

Testosterone is the principal androgen of the testis and belongs to the class of hormones known as steroids. The capacity of the testis to produce

testosterone varies between species and within individuals. Scores of factors, including age, breeding, season, nutritional status and climatic components, affect these functions. All steroid compounds contain a cyclopentano-perhydrophenanthrene nucleus, consisting of a full hydrogenated phenanthrene rings (A, B & C) to which is fused a firm carbon cyclopentano ring (D). There are three basic compounds to which most of the ovarian, testicular and adrenal hormones are related. These are (i) Oestrone an 18 C atom compound, is related to natural oestrogens, (ii) androstane, a 19 C atom compound and is the parent compound of androgens. (iii) pregnane a 21 C atom compound, which is the basic nucleus for adrenal corticoid hormones. Testosterone is a  $\Delta^4$ , 17  $\beta$  -hydroxyzone-androstane. The structural formulae of these compounds are shown in Fig. 100.

The main endocrine function of the androgens is to maintain the integrity of the reproductive tract and the secondary sex characteristics and to keep up the libido of the male.

A summary of physiologic actions of testosterone (Zarrow *et al*, 1961) is as follows.

#### I. SPERMATOGENESIS

1. Inhibition via the pituitary and/or hypothalamus.
2. Direct stimulation of spermatogenesis.
3. Prolongation of epididymal sperm life.

#### II. ACCESSORY GLAND AND TISSUES

1. Stimulation of growth and secretory activity of seminal vesicles, prostate, bulbourethral and preputial glands.

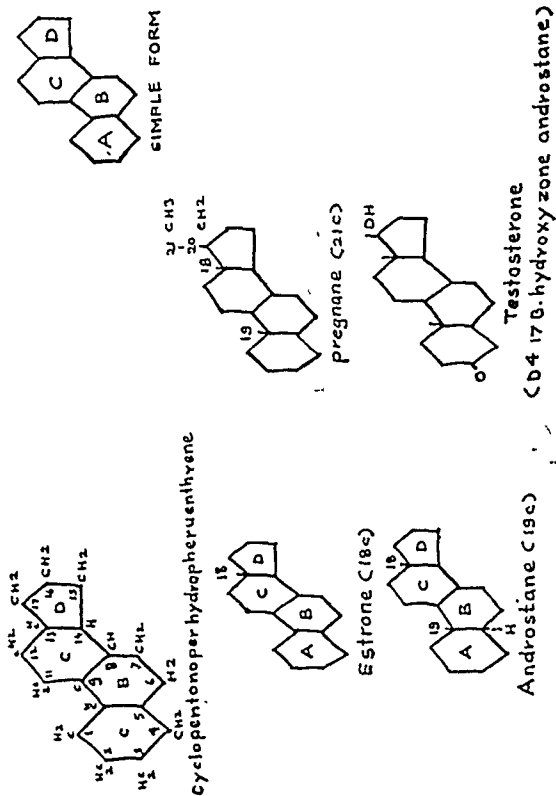


Fig. 100. Structural formulae of different hormones.

2. Stimulation of growth of penis and scrotum.

### III. SECONDARY SEX CHARACTERISTICS

1. Distribution of body hairs.
2. Configuration of body.
3. Comb, wattles, spurs, feathers of birds, claspings pads of amphibia, markings — dorsal spine of certain fishes.
4. Pitch of voice.
5. Behaviour-sexual and aggressive.

### IV. METABOLISM

1. Promotes nitrogen retention, protein, anabolic action, enzyme synthesis.
2. Increase storage of creatine.

Wakeling (1959) reported that like estradiol testosterone causes an increased blood flow and stimulates the growth of the target tissues. Small doses of testosterone inhibits spermatogenesis, presumably acting via the hypothalamus and pituitary. Large doses of androgens stimulate the testis directly. Both motility and life span of epididymal sperms are influenced by testosterone. The hormonal activity of testis influences both aggressive and male sex behaviour (Beach, 1918).

#### Secondary sexual characters

Secondary sexual characters are capable of developing in either sex but normally appearing only in the one which supplies the appropriate hormonal stimulus e.g. beard (Trotter, 1922). development of hump and broad shoulders in male cattle. Ambosexual traits are those common in kind but not always in degree to both sexes, but require the presence of a sex hormone for their normal development e.g. axillary

hair. The male hormones as a group called androgens are mainly responsible for the secondary sex characters in the males. Androgens can also be produced by the ovaries and adrenal cortex. It is thus clear that androgens are not limited to one sex. It is on account of the secretion of excess of androgens by the ovaries and adrenal cortex, a condition known as adrenal virilism is produced in cows. Women may develop facial hairs if adrenals secrete large amounts of androgens.

There are certain secondary sex characters common to all breeds of cattle but some characters are specific to particular breeds.

#### 1. GENERAL APPEARANCE

In general males are bigger than females. This is due to the effect of oestrogens upon the epiphysis of the long bones. The males have a general masculine type expressed in thicker coarser and more rugged features.

#### 2. TEMPERAMENT

Aggressive, energetic, virile and excitable in bulls.

#### 3. LOOK

Aggressive and usually ferocious.

#### 4. VOICE

The voice in males is deeper pitched due to more growth of larynx (Asdell, 1946).

#### 5. MAMMARY GLANDS

These are present as only rudimentary teats.

#### 6. HUMP

The development of hump in bulls is the characteristic feature of Zebu cattle.



## 7. FAT DEPOSITION

In bulls the fat is deposited more anteriorly than in the cows where it is markedly posterior (near hind quarters and udder).

## 8. SKIN

In bulls the skin is not so soft and thin as in cows.

## 9. CARRIAGE

Males are erect, gaunt, proud and alert in carriage.

10 The general traits in bulls include always pawing the earth with their forelegs, goring against any hard structure and tendency to fight against their own sex for herd supremacy.

## 11. CHANGE IN COLOUR, HORNS AND SKULL BONES

Particular secondary sex characters in some breeds include frontal bones more developed and prominently bulged out in the males and less so in cows e.g. in Gir breeds of cattle and Jaffri buffaloes. Horns are thicker at the base, shorter and stumpier and less curving in bulls e.g. Gir cattle.

Dark colour ( $\frac{1}{2}$ " to  $\frac{3}{4}$ " in width round the eyes is seen in bulls of Kankrej breed. [Prominent pendulous sheath is present in bulls of Gir, Sindhi and Sahiwal breeds. The sheath is tight in draft breeds of bulls like, Khilar, Anritmahal and Hallikar].

## Environment and Reproduction

The influence of season, feed supply and other environmental factors is seen more in female reproduction but less changes occur in the male. In wild animals which have restricted breeding seasons, behavioural changes occur in the male during breeding season, refer-

red to as 'rutting'; during the non-breeding season there is cessation of spermatogenesis to a great extent and also in the gonadal hormone production. This regulation is under the influence of pituitary hormones. Strong influences of day light, feed supply and environmental temperature influence the hypothalamic pituitary control mechanism and bring about the gonadal changes. Mares, donkeys, birds, cats and ferrets are long day breeders and sheep, goat and deer are short day breeders.

The most important environmental factor controlling reproductive cycles is length of day light to which the animal is subjected. In birds the effect of light is important in encouraging egg production. In summer months the high testicular temperature leads to increased percent of abnormal sperms in ram semen (Glover, 1956). Increase of temperature in the summer has influence on pituitary thyroid axis. Improved and increased nutrient feed quality and quantity increases the gonadal activity. Hence the practice of 'flushing' in sheep before the commencement of breeding season is beneficial. Another example of adjustment of internal sexual rhythms to external seasonal rhythms is seen when sheep indigenous to one hemisphere adjust their breeding season to the new calendar when they are moved across the equator. Animals in tropical and subtropical areas customarily display less restricted seasons than the animals in temperate zones, showing seasonal reproductive cycles. Because of the assured environmental influence cows and rabbit have got them freed practically from this part of environmental influence. Breeding efficiency declines in summer in areas where high temperature is common. Also there is a depression of pro-

duction of milk and butter fat in summer. With all this information, the knowledge regarding the effects of environmental influences on the neuroendocrine systems of farm animals is far from complete. The environment cannot be controlled efficiently.

### Spermatogenesis

The male pituitary apparently secretes FSH and LH in reasonably constant amounts from day-to-day. The normal bull testis secretes estrogen as well as androgen (Dorfman *et al*, 1935). Feminizing effects of sertoli cell tumour in dogs and man suggest that sertoli cells may be the source of estrogen (Berthrong *et al*, 1949; Huggins and Moulder, 1945). In short, the hormonal control of spermatogenesis in the bull is as follows (Salisbury and VanDemark, 1961).

(1) On reaching puberty the LH gonadotropin acts on leydig cells to produce the male hormone (androgen).

(2) The male hormone conditions the germinal epithelium to respond to FSH.

(3) FSH initiates spermatogenesis.

(4) The process of spermatogenesis is maintained by proper balance of FSH, LH, androgens and estrogen.

Steinberger (1971) proposed that multiplication and growth of gonocytes occurring prenatally, as well as during the first few days after birth and formation of primitive type A spermatogonia are possibly under control of testosterone. The formation of type A and B spermatogonia, formation of primary spermatocytes and progression of the meiotic prophase do not require either gonadotropic or gonadal hormones, however the sequence may be influenc-

ed by growth hormone. The process of reduction division is under the control of testosterone. The early steps of spermatid formation (steps 1-15) may be under no hormonal control or may require testosterone. The late stages of spermatids maturation (steps 15-19) require the presence of FSH.

### Sperm transport and sexual behaviour

In response to visual and tactile stimuli there is a release of oxytocin which plays an important role in sperm transport, with the increased uterine contractions. Natural mating or artificial insemination causes milk ejection and increased uterine contractions. According to Moghissi (1968) sperms migrated through the cervix of extirpated bovine and human uterus when the cervix contained estrual (bovine) or mid-cycle (woman) mucus, but the cells did not migrate beyond the internal os of any tract isolated from neurologic and hormonal influences. Epinephrine inhibits the effect of oxytocin in stimulating both the milk let down and uterine contractions in the cow. Therefore care should be taken to handle the cow while breeding or milking. Hancock and McGroven (1968) stated that any impairment of neurogenic stimuli to myometrial activity can prevent transport of sperms. Spermatozoa obtain energy for maintaining life and motility from anaerobic fructolysis and not aerobic respiration (Mann, 1954). As a result of action of estrogens, the metabolism of female genital tract is largely anaerobic and glycolytic. Motility survival time and fertility of sperms are therefore maximum under anaerobic conditions and are considerably less so under aerobic conditions (Mann, 1954). Under the influence of estrogens the cervical mucus becomes more

liquid and increases in quantity and its sodium chloride content increases, this facilitates sperm survival. The pH becomes more alkaline and glycolytic metabolism becomes more tense, reaching its peak at the time of ovulation. At the same time estrogens increase the activities of such enzymes as alkaline phosphatase, sulfatase, hexokinase and various dehydrogenases, creating conditions which in all probabilities facilitate the capacitation of spermatozoa. Oxytocin, as is well known facilitates sperm transport (Amoroso, 1963).

Hormonal aspects of sexual behaviour have been extensively studied by Beach (1948). Sexual behaviour is required to be understood in relation to physiological functions of the sexual organs, the nervous and the endocrine system. The different patterns of courtship and mating depend on the anatomical structure of the sexual tract and secretions of accessory glands. Sexual behaviour in both the sexes normally act in concert. The different patterns and stimuli of the sex are highly co-ordinated with the other. Copulatory patterns of the male are controlled by complex pathways from the autonomic nervous system. Both erection and ejaculation involve muscular contractions and cortical co-ordination primarily triggered through the sacral autonomic Parasympathetic nerves (Walton, 1955, 1960).

Beach (1952) studied how hormones affect the sex behaviour by early removal of testosterone by castration in the rat at birth. He found that castration interfered with the ability to copulate later in life, even though sufficient testosterone was administered to restore the libido. In the early critical age, testosterone is necessary for the normal growth of the penis. Sexual function is

dependant on the normal endocrine status.

### **Testicular relationship with other endocrine glands**

Hypophysis is a necessary link in the chain of reactions leading to sexual activation. Hormonal interplay by other endocrine glands is also involved in the various processes of male reproduction. Seasonal changes in the thyroid and testis of buffalo have been reported by Bhatnagar and Bhattacharya (1953). The thyroid gland has an indirect if not direct role in the male reproduction. Thyroidectomy of a bull at 4 months age had no effect on spermatogenesis but resulted in complete loss of libido (Petersen *et al*, 1941; Spielman *et al*, 1945). In rams feeding of thyroprotein in the hot weather prevents the drop in spermatozoa production and in the season when sperm production is quite high feeding of thiouracil (an inhibitor of thyroxin secretion) causes a drop in sperm production. This indicates the role of thyroid in sperm production (Bogart and Mayer, 1946). In addition to testicles, adrenal cortex is also capable of producing the androgens. Therefore it is possible that the functions of these glands are interrelated. The androgen secretion from the cortex of the adrenals is increased during adrenal hyperplasia or neoplasia, nymphomania in cattle and adrenal virilism in human. Adrenal cortex has been cited as the source of urinary estrogens in bulls and steers. The urine of the stallions is a rich source of estrogens. Estrogens have been isolated from the testes of stallions, bulls, boars, dogs and man. Schaffenburg and McCullagh (1951) detected estrogen activity in bovine sperms. It is supposed that resorption of stored sperm may contribute estrogens. The

concentration of oestrogens in the urine of mature stallion is 1420 to 3202  $\mu$  g. (oestrone), oestradiol 17  $\beta$  101 to 271, in the bull 0.1 or 0.2 to 0.6 per 100 ml. urine (values for 24 hour excretion) (Velle, 1966). The estrogens and the androgens maintain the regulation of pituitary gonadal axis. Surgical removal of the pituitary gland abolishes the normal cycles in many mammals and render the gonads unable to respond to environmental influences.

Relative activities of pituitary immature rat litre units, is next to lowest in the most prolific species.

Man,	horse,	sheep,	pig,	cow
100	30	2.5	1.5	0.19

Still the exact manner in which the changes in the environment, exercise, temperature, light, food supply and social impact affect the gonadal development and function is not clearly known (Amoroso, 1963).

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# Chapter 33

## Spermatogenesis and morphology of the Spermatozoon

### I. INTRODUCTION

With the rapid development of artificial insemination in domestic animals there has been an increasing interest in the study of spermatogenesis in the last two and half decades. Most of the investigations have remained limited to several laboratory species such as rat, mouse, guinea pig, hamster etc. However, only a few workers have done detailed study on the process of spermatogenesis in domestic animals.

ter divides to give rise to another stem cell and the differentiating cell to maintain the continuous production without exhausting themselves. The differentiating cells are those cells which are at various steps toward specialization, which divide to give rise to other differentiating cells but not the new stem cells. The differentiating cells give rise to primary spermatocytes.

types; spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa.

### A. Spermatogonia

The identification of the types of spermatogonia largely depends on the morphological characteristics of the nucleus. The fixation that precipitates the chromatin to various degrees becomes an important factor in the study of these cells. Usually the Zenker-formol and Bouin's fluid have been used as fixatives. Several types of spermatogonia have been distinguished depending upon their morphological characteristics: the dust like (Regaud, 1901) or A-type (Allen, 1918); intermediate type (Clermont and Leblond, 1953) originating from A-type spermatogonia and the crust like (Regaud, 1901) or B-type (Allen, 1918) spermatogonia.

These three types of spermatogonia are present in the bull (Kramer, 1960; Amann, 1962; Hochereau, 1968; Berndtson and Desjardins, 1974), buffalo bull (Nagesharao *et al*, 1970; Dhingra and Goyal, 1975; Sharma, 1977), stallion (Ellery, 1971), ram (Ortavant, 1956; Gupta, 1972), and boar (Swierstra, 1968).

The development of the gonocytes into A-type spermatogonia involves a transitional stage represented by the pre-spermatogonia or prospermatogonia.

#### (a) PRE OR PROSPERMATOGONIA (Ao Spermatogonia)

The term pre or prospermatogonia (Ao spermatogonia) has been used by several authors to designate the same cell or reserve stem cells (Sapsford, 1962; Hochereau, 1970). In the adult domestic animals, Ao spermatogonia has been described in the bull, (Kra-

mer, 1960; Amann, 1962; Hochereau, 1968), buffalo bull (Dhingra and Goyal, 1965), ram (Sapsford, 1962) and boar (Swierstra, 1968). They are rounded and their chromatin is pale, granular and have one or more nucleoli which are scarcely visible. In the buffalo bull the chromatin is finely granulated and evenly distributed in somewhat darker nucleoplasm. The nucleoli, one or two in number, are situated close to the nuclear envelope (Dhingra and Goyal, 1975). By division Ao spermatogonia give rise either to A<sub>1</sub> or to A<sub>0</sub> spermatogonia and thus termed as prespermatogonia (Hochereau, 1970). Clermont and Bustos (1968) and Clermont (1969) have called them "Reserve Stem Cells".

#### (b) A TYPE SPERMATOGONIA

Several successive generations of A-type spermatogonia which undergo progressive differentiation during spermatogenesis are present in all the species studied. Spermatogonia type-A (Fig. 101) are large cells with ovoid nuclei. The chromatin finely granulated, homogenously scattered in the nucleoplasm has been reported for all mammals by Courot *et al* (1970). The type A<sub>1</sub> spermatogonia in ram contain a well stained nucleolus placed centrally. The type A<sub>2</sub> spermatogonia, contain darker chromatin than type A<sub>1</sub> spermatogonia (Gupta, 1972). In buffalo bull A<sub>2</sub> spermatogonia contain granulated chromatin in the discoid shaped nucleus (Dhingra and Goyal, 1975). During multiplication, Courot *et al* (1970) have described that the metaphase plate of the dividing A-type spermatogonia are frequently perpendicular to the seminiferous tubule wall, whereas Gupta (1972) has observed this phenomenon only in the degenerating spermatogonia during nonbreeding season in the ram.

### (c) INTERMEDIATE TYPE SPERMATOGONIA

The intermediate type spermatogonia are smaller than type-A spermatogonia (Fig. 102). Berndstein and Desjardins (1974) have described that in these intermediate type spermatogonia in the bull, clumps of chromatin adhere to the nuclear membrane and the nucleolus is smaller than that of type-A spermatogonia. In the buffalo bull the chromatin is dispersed and the nucleolus is central in position (Dhingra and Goyal, 1975). In the ram, 2 or 3 nucleoli are present in the ovoid nucleus having coarse granular chromatin (Gupta, 1972).

### (d) B TYPE SPERMATOGONIA

The type B spermatogonia are comparatively smaller in size and characterized by almost spherical nuclei containing large dark granules of chromatin located adjacent to the nuclear membrane, containing 2 or 3 nucleoli (Fig. 103) in the ram (Gupta, 1972). In the bull and boar the chromatin granules are scarce (Ortavant *et al*, 1969). The clumping of the chromatin which adheres to the nucleolus and also along the nuclear membrane of the type B spermatogonia of buffalo bull with more or less central location of the nucleolus has been described by Dhingra and Goyal (1975) and Sharma (1977). A similar chromatin pattern for type B spermatogonia of bull has also been described by Berndstein and Desjardins (1974).

proginator cells (Fig. 103) the nuclear diameter being slightly smaller. These cells have distinct chromatin pattern. The nuclear membrane is usually less distinct than that of type B spermatogonia. They have sometimes been called "resting spermatocytes" but since their nuclei synthesize DNA preceding the first maturation division (Swift, 1950) and therefore are only apparently resting. This term is, therefore inaccurate since the synthesis of DNA and other compounds take place during this phase. The term preleptotene spermatocyte used by Tobias (1956) is now widely accepted. It is at this stage that the labelled compounds like thymidine  $3H$  and  $32P$  gets incorporated in the DNA used for the study of duration of spermatogenesis.

As these cells develop, the chromatin crusts or granules resolve into finely beaded filaments and then the chromatin become clearly filamentous, and takes the morphological configuration of leptotene step of the prophase of the first meiotic division. These filaments are located centrally in the nucleus and forms a ball like structure and the nucleus increases in size (Fig. 104). The DNA synthesis goes on during the beginning of leptotene stage and ends in the sex chromosomes just before spiralization which is incompatible with duplication of DNA. A late leptotene stage spermatocyte contains a tetraploid amount of DNA in the bull.



As the nucleus volume progressively increases the chromosomes become more contracted and thicker and enter the pachytene step (Fig. 103). The chromatin in this condition stays for a longer period of time, during which the nuclear and cell volume progressively increase. This is followed by a short diplotene step (Fig. 104) during which the chromosome partly splits and is characterized by the maximum size of the nucleus, dispersion of the chromosome and formation of tetrads between analogous chromosomes. Finally, it enters the diakinesis phase which is very transient during which the pairs of highly spiralized chromosomes are no longer attached except by the chiasma. It is extremely rapid in the ram and bull (Courot *et al*, 1970). Finally the nucleus goes through metaphase, anaphase, and telophase of the first maturation (meiotic) division to yield two diploid cells, the secondary spermatocytes.

#### (b) SECONDARY SPERMATOCYTES

The nucleus of the secondary spermatocytes contain dark thin net work of chromatin. The secondary spermatocytes remain in interphase only very briefly and therefore are seldom encountered in sections of seminiferous tubules. They very soon go into the second maturation division, then each spermatocyte divides to give rise to two spermatids containing haploid number of chromosomes.

#### (c) SPERMATIDS

The nuclei of the spermatids resemble those of the secondary spermatocytes but are smaller (Fig. 102). The spermatids undergo a series of complex changes called spermiogenesis leading to the production of the highly differentiated germ cells, the spermatozoa.

### NUCLEAR CHANGES

The nuclei of the newly formed spermatids are rounded and become elongated during the middle of spermiogenesis and then assume the shape of the head of spermatozoa which is roughly oval and flattened in the domestic animals whereas in the rodents it is hook-like in shape, and is pear-shaped in man.

The nuclear membrane of the developing spermatid is double and contain a variable amount of space between them. At the region of the acrosomic vacuole the space becomes narrow and the membrane is thickened on the internal surface opposite the acrosome and on its external surface at the level of the socket (Sapsford *et al*, 1967). A few nuclear pores have been observed in the developing spermatids the role of which is still uncertain. The nucleus of the young spermatid contain several large granules of DNA of various sizes scattered on a filamentous network. During the elongation phase, the uniformly distributed DNA accumulates at the base of the head after its narrowing in the ram (Esnault *et al*, 1964).

### CYTOPLASMIC CHANGES

The spermatozoon is formed as a result of metamorphosis of the spermatids. When the spermatozoon is ready to be released from the seminiferous tubule it contains a highly elaborated cytoplasmic constituent, the protoplasmic droplet. During the process of metamorphosis a large part of the cytoplasm from the spermatid is eliminated in the form of a residual body.

This development process is divided into 4 phases: Golgi phase, cap phase, acrosome phase and maturation phase (Leblond and Clermont, 1952b).

1. *Golgi phase*: The starting point of transformation of the spermatid is marked by the appearance of the pro-acrosomic granules in the Golgi region. These granules coalesce together in a single acrosomic granule which are now contained in a single vesicle and is located close to the nucleus of the young spermatids.

2. *Cap phase*: During this phase the vesicle extends in size and the acrosomic granule assumes a spherical shape and flattens on the nucleus of spermatid.

3. *Acrosomal phase*: During this phase the granule extends further and occupies all of the vesicle which is now called "the acrosome". The acrosome extends further over the surface of nucleus as it undergoes elongation. The nucleus and the acrosome become oriented towards the nuclei of the Sertoli cells and come in contact with the cell membrane (Fawcett, 1965). This takes place through the undulations of the cytoplasmic membrane of the Sertoli cells (Clermont, 1961).

4. *Maturation phase*: During this phase the nuclear changes still predominate. The nucleus gradually acquires the shape typical of each species. At the same time the karyoplasm becomes condensed in coarse granules which fuse together to form a dense homogenous mass with no discernable structure.

While the nucleus is elongating and condensing the outline of the tail of the future spermatozoan appears and develops. During the early phases a filament prolongs from one of the centrioles and projects at random from the cell while remaining surrounded by the cytoplasm (Leblond and Clermont,

1952B; Sapsford *et al.*, 1967). Just before the beginning of the spermatid elongation the centrioles place themselves against the nuclear membrane and the formation of implantation plates and striated columns can be seen. At about the same time cytoplasmic microtubules arise and become laterally associated to form a roughly cylindrical structure, called the caudal sheath or Manchette (Burgos and Fawcett, 1955). This extends caudally from a ring like specialization of the cell membrane at the posterior margin of the acrosomal cap. At this time there is marked elongation of the spermatid, so that the bulk of the cytoplasm is displaced caudally where it surrounds the posterior part of the axonema (flagellum). The flagellum at this time consists only of axial filament complex (axonema), with two central fibrils and nine peripheral doublets that are continuous with the wall of the distal centriole. The centriole is encircled by a ring called the annulus (Burgos *et al.*, 1970). As the tail gets further differentiated, nine longitudinally oriented segmental columns arise around the centriole and are joined to each other proximally and to the base of the nucleus to constitute the connecting piece. Distally the nine structural elements which form the connecting piece are joined to nine thick longitudinal fibres which develop peripheral to the doublets of the axial filament complex. The smaller dense ring fix to the flagellum remains and in its further elongation it is carried distally. As it moves back the manchette disappears and mitochondria gather around the segment of flagellum and, wrap helically around it to complete the differentiation of the middle piece. During its further development a 'succession of

circumferentially oriented ribs are deposited around the tail fibres posterior to the annulus to form the fibrous sheath of the tail. With the completion of the differentiation of the tail the excess cytoplasm is excluded as residual body.

#### D Sertoli Cells

These cells are located along the basement membrane of the seminiferous tubules. The cytoplasm protrudes towards the lumen of the tubule and resembles an arborescence with trunk where the nucleus is located resting on the seminiferous tubule wall. The nucleus of Sertoli cell is very irregular in shape and contains fine chromatin granules. There are one or several nucleoli with an appearance varying according to species: multivesicular in the ram and the bull (Nicander, 1963). The general shape and position of the nucleus vary in relation to the stage of seminiferous epithelial cycle. Their number however remain unchanged and in fact these cells no longer divide in mammals once they are differentiated. The Sertoli cells provide mechanical support and protection for the developing germ cells and it is not unlikely that they may participate importantly in their nutrition. The close relationship of the germ cells with Sertoli cells suggest that the Sertoli cells control the exchanges in and between the germ cells (Roosen-Runge, 1962). The Sertoli cells also have considerable phagocytic activity besides the normal resorption of the residual bodies under pathological or experimental conditions they can resorb the germ cells undergoing degenerations (Roosen-Runge, 1975; Lacy, 1970; Higon and Böhgers, 1964, 1966). Sertoli cells also play a particular role in the architecture of seminiferous epithelium and are concerned with the

release of the spermatozoon (Leblond and Clermont 1952b; Burgos and Vitable 1964) and the resorption of residual bodies (Clermont and Percy (1957), Courot 1962; Attal and Courot 1963).

### III. CYCLE OF THE SEMINIFEROUS EPITHELIUM

The cycle of the seminiferous epithelium can be defined as the complete series of changes occurring in a given area of the seminiferous epithelium between the two successive appearances of the same cellular association. The cycle has been subdivided into various stages. Several methods for classifying the seminiferous epithelial cycle stages have been proposed two of them are now in use: one is based on the morphology and cytology of germinal cells and their relative position within the seminiferous tubule (Roosen-Runge and Giesel 1950; Ortavant 1958; Swierstra 1968; Courot *et al.*, 1970; Foote *et al.*, 1972; Gupta 1972; Sharma and Gupta 1977). The other is based on the development of the acrosomic system of the spermatid (Leblond and Clermont 1952b).

#### A. Staging based on the morphological development of the germ cells and cellular association

Using the classification based on the morphological development and cellular association of the germ cells the seminiferous epithelial cycle (Table 39) has been divided into 8 stages in ram (Ortavant 1958), bull (Ortavant 1959; Amann 1962), boar (Swierstra 1968), dog (Foote *et al.* 1972), stallion (Swierstra *et al.*, 1971) and buffalo bull (Sharma and Gupta 1977). Following the similar approach the seminiferous epithelial cycle has been divided into 6 stages for the human (Clermont 1963).

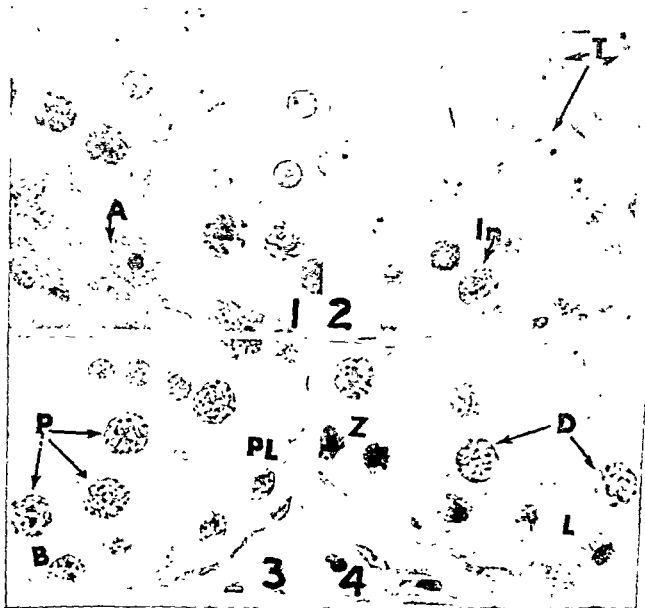


Fig. 101. Spermatogonium type A(A) showing a well stained centrally placed nucleolus in the ram (H & E)  $\times 1000$ .

Fig. 102. Spermatogonium Intermediate type (In) in the ram. The nucleus contains coarser chromatin granules. Spermatid (T) containing several nuclei (H & E)  $\times 1000$ .

Fig. 103. Spermatogonium type B(B) in the ram containing dark large chromatin granules and several nuclei. Preleptotene phase of primary spermatocyte (PL). Note the appearance of fine threads of chromatin. Pachytene phase of primary spermatocyte (P). The chromatin appears more contracted and thicker (H & E)  $\times 1600$ .

Fig. 104. Leptotene (L), Zygotene (Z) and Diplotene (D). Note the ball-shaped appearance of fine threads, bouquet like arrangement, and small irregular short rods of chromatin in the three phases respectively (H & E)  $\times 1600$ .

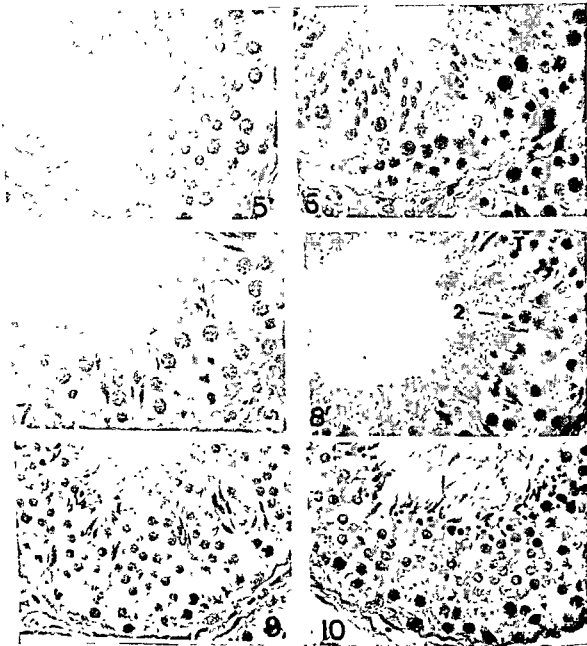


Fig. 105-110. Cross sections of seminiferous tubules showing the stages of the seminiferous epithelial cycle in the ram (H & E)  $\times 800$ .

Fig. 105. Stage 1: One generation of spermatids which are round, are present.

Fig. 106. Stage 2: The spermatids have started elongating.

Fig. 107. Stage 3: The spermatids have become more elongated and are grouped in bundles.

Fig. 108. Stage 4: Older generation of primary spermatocytes have entered meiotic division. Secondary spermatocytes (2) and newly formed spermatids (T) are seen.

Fig. 109. Stage 5: Two generations of spermatids are present. One generation is composed of newly formed spermatids with spherical nuclei while the older second generation of spermatids are seen penetrating deep towards the basement membrane.

Fig. 110. Stage 6: The older generation of the spermatids line the lumen of the tubule and are about to be released.

Table 39

## COMPARISON OF THE CLASSIFICATION OF THE STAGES OF THE CYCLE OF SEMINIFEROUS EPITHELIUM IN DOMESTIC ANIMALS AND MAN

Authors	Year	Species	STAGES							
			Spermatid elongation		Meiotic division				Spermatozoa release	
Ortavant	1958	Ram	1	2	3	4	5	6	7	8
Ortavant	1959	Bull	1	2	3	4	5	6	7	8
Ortavant	1959	Boar	1	2	3	4	5	6	7	8
Amann	1962	Bull	1	2	3	4	5	6	7	8
Henricson and										
Backstrom	1963	Boar	1	2	2	3	4	—	5	6
Clermont	1963	Man	3	4	5	6	—	1	—	2
Swierstra	1969	Boar	1	2	3	4	5	6	7	8
Gupta	1972	Ram	3	4	5	6	—	1	—	2
Ellery	1971	Stallion	3	4	5	6	—	1	—	2
Foote <i>et al.</i> ,	1972	Dog	1	2	3	4	5	6	7	8
Swierstra	1974	Stallion	1	2	3	4	5	6	7	8
Sharma and Gupta	1977	Buffalo Bull	1	2	3	4	5	6	7	8

boar (Henrickson and Backstrom, 1963), stallion (Ellery, 1971) and ram (Gupta, 1972). According to Sharma and Gupta (1977) following eight stages may be defined in the seminiferous epithelial cycle of the buffalo bull.

*Stage 1:* This stage extends from the complete disappearance of the spermatozoa from the lumen to the beginning of the elongation of the spermatid nuclei (Fig. 105).

*Stage 2:* This stage extends from the beginning of the elongation of the spermatid nuclei to the onset of distinct bundle formation of the young spermatids (Fig. 106).

*Stage 3:* This stage extends from the beginning of the bundle formation of the young spermatids nuclei the start of the first maturation division of primary spermatocytes (Fig. 107).

*Stage 4:* This stage extends from the beginning of first maturation division to the end of second maturation division and is characterized by the initial appearance of Intermediate type spermatogonia (Fig. 108).

*Stage 5:* This stage extends from the end of the second maturation division to the initial appearance of B type spermatogonia (Fig. 109).

*Stage 6:* This stage extends from the initial appearance of B type spermatogonia to the time when all bundles of elongated spermatids start migrating towards the lumen of seminiferous tubule.

*Stage 7:* This stage extends from the time when all bundles of elongated spermatids start migrating towards the lumen until all reach the lumen.

*Stage 8:* This stage extends from the time when old spermatids or spermatozoa line the luminal surface of the germinal epithelium until their complete disappearance from the lumen (Fig. 110).

The steps involved in the morphological differentiation of the spermatid particularly at stage 5 as described by Roosen-Runge and Giesel (1950), Ortavant (1958, 1959) and Courot *et al* (1970), seems not to have been identifiable by other workers (Amann, 1962; Swierstra, 1968; Swierstra *et al*, 1974; Sharma and Gupta, 1977). Therefore, these authors have used a different criteria for the identification of stage 5. This may be due to difference in the fixative and staining procedures used, as the chromatin pattern varies depending upon the fixative used (Clermont, 1966).

#### **B. Classification based on the development of acrosomic system (spermeiogenesis)**

Histological section stained with Periodic Acid Schiff (PAS) reagent and counter-stained with Harris haematoxylin are used to study the process of the development of acrosomic system. From a morphological point of view, several stages are distinguishable which are used as a basis for the classification of seminiferous epithelial cycle (Clermont and Leblond, 1955; Gupta, 1972). The following 14 stages are described in ram by Gupta (1972) following the method described by Clermont and Leblond (1955). The process of spermiogenesis of the ram is divided into 4 phases consisting of 14 stages (Fig. 111). These stages are designated by Roman numerals.

1. Golgi phase — I, II, III stages
2. Cap phase — IV, V, VI, VII stages
3. Acrosome phase — VIII, IX, X, XI, XII, stages
4. Maturation phase — XIII, XIV stages.

#### **GOLGI PHASE**

*Stage I:* This is characterized by the presence of slightly stained spherical zone called the idiosome, near the nucleus of the newly formed young spermatid.

*Stage II:* This is characterized by the presence of PAS positive particles, the proacrosomic granules, in the idiosomes.

*Stage III:* This is characterized by the formation of a single rounded granule, the acrosomic granule.

#### **CAP PHASE**

*Stage IV:* This is characterized by an increase in the size of the acrosomic granule which flattens lightly at the surface of the spermatid nucleus.

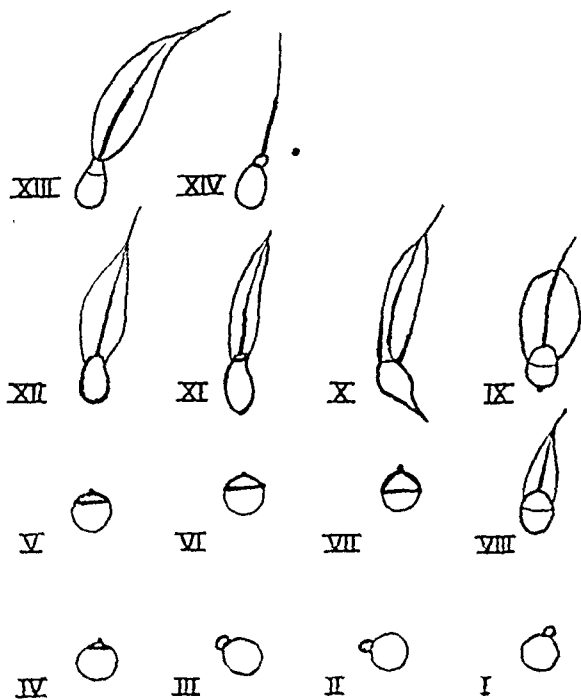
*Stage V:* This is characterized by the appearance of the head cap which extends slightly on either side of the acrosomic granule.

*Stage VI:* This is characterized when the head cap extends further on either side and cover about one-third of the nuclear surface.

*Stage VII:* This is characterized when the head cap covers about one half of the nuclear surface.

#### **ACROSOME PHASE**

*Stage VIII:* During this stage the spermatids with their developing acrosomic system become oriented towards the basement membrane of the seminiferous tubule. The acrosomic granule and the head cap slightly protrudes





anteriorly which is now called "acrosome".

*Stage IX:* During this stage the acrosome enlarges and protrudes further at the tip of the nucleus. The nucleus of the spermatid also becomes elongated.

*Stage X:* During this stage the acrosome becomes elongated, extending apically. The spermatids enlarge further and flatten slightly. The acrosome now becomes continuous with the head cap.

*Stage XI:* During this stage the acrosome becomes triangular in appearance at the anterior end of the developing spermatid. The spermatid is now more flattened.

*Stage XII:* During this stage the acrosome assumes a crust or crescent shape at the anterior end of the spermatid nucleus which now appears paddle-shape.

#### MATURATION PHASE

*Stage XIII:* During this stage the acrosome flattens at the apex of the head cap. A decrease in PAS reactivity is also observed.

*Stage XIV:* This is the final stage of spermiogenesis. The acrosomic system (head cap and acrosome) are faintly stained. At the neck region a cytoplasmic droplet is present whereas most of the cytoplasm gets detached. The cells now called spermatozoa are ready to be released.

#### IV. RELATIVE FREQUENCY AND DURATION OF THE STAGE OF THE CYCLE OF SEMINIFEROUS TUBULE

For given species the relative frequency of the stages is constant. This

constancy is observed in different sites of testis, between testis of same animals and among animals of same species (Amann, 1962; Hochereau, 1963; Swierstra, 1968; Gupta, 1972; Sharma and Gupta, 1977). A significant change in the frequencies of stages of the seminiferous epithelial cycle has been observed by Gupta (1972) during the non-breeding season in the ram. The frequencies of the stages expressed in percentage, correspond to the relative duration of the various stages. Thus in a random section through the seminiferous tubule, stages of short duration appear rarely while as stages of longer duration are seen frequently (Table 40).

Two methods have been used for the study of duration of the cycle of seminiferous epithelium. One is based on blocking spermatogenesis by harmful agents (Heat, X-irradiation) and the other using the labelled compound ( $p^{32}$ , Thymidine  $^3H$ ) which gets incorporated into the DNA. When a proper dose of X-rays is given it destroys a large percentage of spermatogonia, which results in a progressive disappearance of spermatocytes and spermatids from the seminiferous epithelium. The rate at which the germ cells disappear was translated in terms of rate of spermatogenesis. Thymidine  $^3H$  has been used for the study of duration of the process of spermatogenesis in bull (Hochereau *et al*, 1964), boar (Swierstra, 1968), dog (Foote *et al*, 1972), stallion (Swierstra *et al*, 1974) and buffalo-bull (Sharma and Gupta, 1977). However, Ortavant (1958) used  $p^{32}$  for the study of the duration of the process of spermatogenesis in the ram. Thymidine  $^3H$  is incorporated in the nuclei of the young spermatocytes at the preleptotene and early leptotene stages. No older

Table 40  
 RELATIVE FREQUENCIES OF THE STAGE OF THE SEMINIFEROUS EPITHELIAL CYCLE IN DOMESTIC ANIMALS

Species	Ram	Boar	Bull	Dog	Stallion	Buffalo-bull
Author	Ortavant (1958)	Swierstra (1968)	Hochereau (1965)	Footo <i>et al</i> (1972)	Swierstra <i>et al</i> (1974)	Sharma and Gupta (1977)
Stage	%	%	%	%	%	%
1	25.0	21.7	10.8	30.8	21.9	16.9
2		10.6	14.4	9.1	12.7	14.9
3	13.7	18.4	3.5	20.1	2.8	15.5
4	21.7	10.5	11.6	12.8	11.5	3.2
5		4.2	8.9	1.6	8.3	15.8
6	10.9	13.1	20.3	5.9	15.4	7.4
7	18.2	10.8	7	8.1	13.3	13.5
8	10.6	10.3	12.0	11.6	14.0	12.6
						15.7
						8
						7
						6
						5
						4
						3
						2
						1
						28.7

spermatocyte and no spermatid shows DNA labelling thus the young spermatocytes form the most advanced labelled cells from where the process of spermatogenesis is followed. Duration of one cycle of the seminiferous epithelium is determined by the progress made by the germinal cells after the uptake of the labelled compound. The whole process of spermatogenesis has been calculated taking into consideration the duration of one epithelial cycle and the number of cycles required. For complete process of spermatogenesis an approximate value of 19 days in the ram (Ortavant, 1958), 51 days in the bull (Hochereau *et al*, 1964), 34.1 days in the boar (Swierstra, 1968), 19 days in the stallion (Swierstra *et al*, 1974) and 38 days in the buffalo bull (Sharma and Gupta, 1977) has been calculated (Table 41).

#### A. Cellular Association and Co-ordination of evolution of germ cells

Table 41  
DURATION (IN DAYS) OF THE SEMINIFEROUS EPITHELIAL CYCLE STAGES IN DOMESTIC ANIMALS

Stage	Buffalo-Bull Sharma and Gupta, 1977	Ram Ortavant, 1958	Bull Hocheau, 1963	Boar Swierstra 1968	Dog Foote <i>et al</i> , 1972	Stallion Swierstra <i>et al</i> , 1974
1	2.4	2.2	4.2	0.9	3.0	2.1
2	1.4	1.1	1.2	1.2	1.7	1.8
3	1.7	1.9	2.7	0.3	0.4	0.4
4	0.8	1.1	1.7	1.0	1.6	1.9
5	0.2	0.4	0.2	0.8	1.1	0.9
6	0.6	1.3	0.8	1.7	2.1	1.6
7	0.7	1.1	1.1	1.6	1.8	1.5
8	0.8	1.0	1.7	1.0	1.9	1.9
Duration (in days) of the seminiferous epithelial cycle (Approx.)	8.6	10.4	13.5	8.6	12.6	12.2
Duration (in days) of the spermatogenesis process (Approx.)	38	49	54	34.4	--	49

Table 42  
SEMIFEROUS EPITHELIAL CYCLE WITH CELLULAR ASSOCIATION  
CHARACTERISTICS OF EACH STAGE IN BUFFALO-BULLS

	Stage 3	Stage 4	Stage 5	Stage 6	Stage 7	Stage 8
A	Spg A Z	Spg A Spg In P	Spg A Spg B P	Spg A Spg B P	Spg A Spg B P	Spg A Spg B P
thou 44	DK Spg Bundle formation	Spg II Spg migration towards base- ment membrane starts	Spi Round Spg Penetrating deep towards basement membrane	Spi Round Spg Centripetal movement starts	Spi Round Spg Centripetal movement ends	Spi Round SPZ ready for release

I, II, A, B, F, In Intermediate spermatogonia, Spg B = B spermatogonia  
Spg A, Spg In, L Leptotene, Z = Zygotene, P = Pachytene,  
DK, Diakinesis, Spg II = Secondary spermatocytes, Spi = Spermatid SPZ = Spermatozoa

However, Ellery (1971) has reported the overshadowing in the typical cell associations by an apparent mixing of another cell association in the same tubular section and by the frequent absence of one or more generations of germ cells. Similar abrasions in the cellular association have also been reported by Clermont (1963) in man.

Sertoli cells may play a role in co-ordinating the evolution of generations of germ cells through phagocytosis of the residual bodies left in place upon the release of spermatozoa (Cleland, 1951; Lacy, 1960; Roosen-Runge, 1962 and Kramer *et al.*, 1964). The co-ordinating factor which acts through the Sertoli cells arises from the primary spermatocytes which in turn would have an impact on the other steps of spermatogenesis (Roosen Runge, 1962). This local mechanism also explains the continuity of the succession of the stages of seminiferous epithelial cycle along the

but takes place along the length of the seminiferous tubule as well (Perry *et al*, 1961). Thus a portion of the tubule displaying one type of cellular association is followed by a portion of the tubule displaying the stage immediately preceding or following in the seminiferous epithelial cycle. There is a continuity of the segmental order (Perry *et al* 1961). Each complete serial series of cellular associations is called spermatogenic wave. However existence of the irregularities of the wave named as modulations have been observed in the rat (Perry *et al*, 1961; Hochereau 1963). The tubular length occupied by one stage of the seminiferous epithelial cycle is not constant as a result variations in the length of the spermatogenic wave have been observed. Therefore the relative ratio of its mean segmental length correspond to its relative frequency (i.e. to their duration) determined on cross sectioned tubules (Perry *et al*, 1961).

### C Mode of Renewal of spermatogonia

The continuous production of spermatozoa by the seminiferous tubules depends on the renewal of spermatogonial population. During each cycle new spermatogonia must appear to replace those which develop into spermatogenic series. Several workers identify A type spermatogonia as stem cells. The type A spermatogonium before its division and differentiation into intermediate type spermatogonium give rise to a stem cell called the Reserve stem cell. The evolution of spermatogenic process is uninterrupted after the establishment of puberty. This continuity is provided by the cyclic renewal of stem spermatogonia all through the animal life. Each of them give rise to a spermatogenic line so the number of stem spermatogonia of a given animal initially conditions its

gamete production. Ortavant (1958) and Gupta (1972) have demonstrated the existence of 5 peaks of spermatogonial mitosis in the ram. The first two are mitosis of type A spermatogonia, the third is the division of intermediate type spermatogonia and last two are the divisions of two generations of type B spermatogonia leading to the production of spermatocytes (Fig. 112). Thus renewal of spermatogonial stem cells takes place by means of bivalent mitosis of type A spermatogonia. Ortavant (1959) also suggested a similar mode of renewal for the bull. Kramer *et al*, (1964) confirmed the existence of the five peaks of spermatogonial mitosis in the bull. They however expressed the view

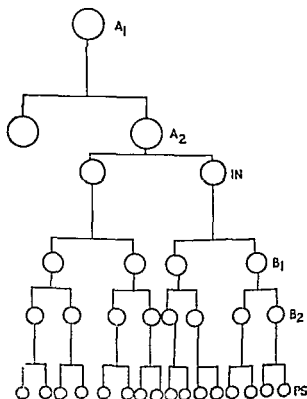


Fig. 112 Diagrammatic representation of the spermatogonial division in the ram showing that the spermatogonia divides four times to produce 16 primary spermatocytes and the other cell becomes a dormant cell.  $A_1 = A_1$  Spermatogonia  $A_2 = A_1$  Spermatogonia  $IN =$  Intermediate type spermatogonia  $B_1 = B_1$  Spermatogonia  $B_2 = B_2$  Spermatogonia  $PS =$  Primary spermatocyte

Table 43  
COMPARATIVE SIZE OF THE SPERMATOOZOA IN DOMESTIC ANIMALS

Head		Middle piece		Main piece		Reference
Length ( $\mu$ )	Width ( $\mu$ )	Length $\mu$	Width $\mu$	Length ( $\mu$ )	Width ( $\mu$ )	
9.15	4.25	14.84	0.670	45.50	0.5	Bonadonna <i>et al</i> (1953)
9.22	5.27	—	—	—	—	Tomar <i>et al</i> (1964)
7.40	4.48	12.41	—	43.61	—	Venkatwami and Vedanayagani (1962)
8.43	4.92	13.11	—	47.81	—	Sharma and Gupta (1977)
7.85	4.03	11.10	—	42.51	—	Osman (1973)
7.00	3.91	9.63	—	43.00	—	Mishikawa <i>et al</i> (1957)
5.86	3.42	7.38	—	35.62	—	Khan (1971)
Rathore		—	—	—	—	Rathore (1961)
8.5	4.25	10.00	—	30.00	—	Hancock (1957)

that spermatogonia (type A) could be have in a variable manner and would be under the influence of some undefined coordinating factor. Amann (1962) suggests that the formation of new stem cells takes place after bivalent mitosis of type A<sub>1</sub> spermatogonia in the bull. However, the division of type A, intermediate type, and type B spermatogonia were staggered over several stages of the cycle and not synchronized, in contradiction to the findings of Ortavant (1959) and Hochereau (1968). Hochereau-de-Reviere (1970) demonstrated the existence of six consecutive peaks of spermatogonial mitosis. The first three mitosis were division of type A spermatogonia.

## V. MORPHOLOGY OF THE SPERMATOZOON

The mature spermatozoon consists of three main parts: the head, the neck, and the tail. The latter is composed of middle piece, the principal piece and the end piece. The dimensions of each of these main parts of the spermatozoon of the domestic animals are given in Table 43.

### A. Sperm Head

The shape of the head in most of the domestic animals is flattened and ovoid. Whereas in most of the rodents it is asymmetrical and hook-like in shape and it is pear-shaped in man. The components of the head are; the nucleus, the acrosome and the post-acrosomal sheath.

### NUCLEUS

During the development of the spermatozoon, its nucleus acquires a shape characteristic of each species (Fig. 113). The nucleus is composed of densely packed chromatin material with the exception of occasional small random

vacuoles. In the caudo-lateral region the chromatin is more dispersed in the nucleus (Heath and Gupta, 1976). The chromatin consists of 43% DNA and 57% arginine-rich proteins (Leuchtenberger, 1956). Sperm contain only half the amount of DNA present in the somatic cells of the same species. Two kinds of sperm are formed: those carrying the X chromosome produce female embryos and those carrying Y chromosome produce males.

### ACROSOME

The anterior part of the nucleus is covered by the acrosome which extends to about two-thirds of the nucleus. The acrosome is clearly divisible into apical, main and equatorial segments. About three quarter of the acrosome is the main segment. The apical segment consists of a ventrally projecting apical ridge which is not as well developed in buffalo sperm as seen in the spermatozoa of *Bos taurus* (Heath and Gupta, 1976). The acrosomal matrix of the main segment is homogenous in appearance except for dense laminar profiles just inside the outer acrosomal membrane of the apical segment (Fig. 113). The matrix of the equatorial segment is more dense. The equatorial segment is clearly distinguishable due to its lesser thickness (Fig. 114). The length of the equatorial segment is unusually long in boar spermatozoa (Nicander and Bane, 1962). Chemical analysis of the acrosomal contents have established the presence of galactose, mannose, fucose, galactosamine, glucosamine and sialic acid (Hartree and Srivastava, 1965). Hyaluronidase enzyme in the sperm head has been demonstrated by Rowland (1914). The acrosomal contents have also been shown to contain several acid hydrolases including acid phos-

phatase,  $\beta$  glucuronidase, N-acetylglucosaminidase (Allison and Hartree, 1970; Dott and Dingle, 1968) and a trypsin-like protease called acrosin or acrosomase (Zaneveld *et al*, 1969). These findings have lead to the interpretation of the acrosome as a highly specialized lysosome. The release of the enzyme during fertilization process is believed to disperse the cells of cumulus and facilitate access of the sperm head to the zona pellucida.

A cone shaped area between the acrosomal cap and the nucleus, the subacrosomal space is present at the apex of the nucleus (Fig. 113). This is not an empty space rather it appears to be a layer or sheath formed by the cytoplasmic matrix (Hadek, 1969). It is located between the nucleus and the acrosome anteriorly and in its posterior aspect between the nucleus and the cell membrane. This has been referred to as "Perforatorium" for the rat and mice spermatozoa but its mechanical function in sperm penetration has not yet been demonstrated.

### POST ACROSOMAL SHEATH

The plasma membrane in the post acrosomal region has the usual trilamellar appearance and contain the dense material. This is now called postacrosomal sheath (Fig. 114). This corresponds to the structure formerly called the postnuclear cap. It is believed that at this region (behind the posterior margin of the acrosome) the attachment and fusion of the sperm and egg membranes take place (Barros and Franklin, 1968; Yanagimachi and Noda, 1970).

### NECK

The neck is situated between the head and first mitochondrial spiral of

the middle piece. In its cranial aspect, the neck is limited by a modified section of the nuclear membrane, the basal plate, which lines the fossa and provides attachment for a large number of fine filaments that extend into it from the articular surface of the connecting piece. The connecting piece comprises of two distinct major striated columns, two minor striated columns and a poorly developed capitulum (Fig. 115). The first dense band of each major striated column is in close alignment with the cytoplasmic coat of dense material on the lateral aspect of basal plate (Fig. 115). Outer dense fibres are also seen in continuity with all the striated columns of the connecting piece. Two outer dense fibres are in direct contact with each minor striated column. The two central axonema are also seen to arise dorsally and ventrally near the minor striated column (Fig. 115).

Proximal centriole is obliquely situated to the long axis of the spermatozoa. Varying amount of dense material is seen between and along side of the nine triplets of the proximal centriole. Laterally some of this dense material is continuous with the dense bands of the striated columns (Heath and Gupta, 1976).

### TAIL

#### Middle piece

The middle piece constitutes the proximal part of the tail. In transverse section it consists of mitochondrial sheath, outer dense fibres and the axonema. The axonema consists of the complex of hollow fibrils characteristic of all flagella, two single fibrils in the centre and nine doublets around them. The axial filament complex is surrounded by



a row of nine outer dense fibres that arise in the neck as caudal continuation of nine segmented columns that forms the wall of the connecting piece. Proximal satellite fibrils are also seen in association with the outer dense fibres. The middle piece distally narrows and the outer dense fibres are now in closer association with their respective axonemal doublets (Fig. 117, 118). The outer dense fibres, 1, 5 and 6 are the largest and distally they appear to flatten (Heath and Gupta, 1976). The termination of the middle piece is clearly demarcated by the end of mitochondrial helix (Fig. 116) and by the presence of a two-part annulus (Fig. 116).

### Principal piece

The principal piece is the longest part of the spermatozoon. It is surrounded by a stout fibrous cover that replaces the mitochondrial layer. It seems to arise from the annulus (Heath and Gupta, 1976). This fibrous sheath is composed of two parallel running longitudinal columns on opposite side of the tail, which are joined together by a series of transverse ribs connecting to the longitudinal columns distally. Proximally the longitudinal columns are continuous with the matrix of outer dense fibres 3 and 8 (Fig. 119). Distally however, fibres 3 and 8 are continued as distinct small dense fibrils (Fig. 120). The principal piece tapers gradually towards the end piece. The junction between the principal piece and end piece is clearly demarcated by the termination of the fibrous sheath (Fig. 121). In the short terminal portion of the tail or end piece, the filament is not surrounded by a sheath and composed of two central and nine peripheral double fibres and the outer nine fibrils are absent.

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# Chapter 34

## Puberty and Sexual Maturity in the Male

### I. PUBERTY

Puberty may be defined as the age or time at which the reproductive organs become functional. In males, puberty is manifested by the development of secondary sexual characters. The sexual maturity is indicated by the presence of mature spermatozoa which corresponds with the period of first sexual interest as indicated by the willingness of the male to mount a female in heat. Sexual interest (sex desire) is the normal instinct in the males reaching puberty. When puberty sets in the male, animals develop the desire to mount over other animals and under favourable conditions they try to protrude the penis out of their sheath and try to copulate. The behaviour of the male at puberty, therefore, is more distinct towards the opposite sex. Sex libido is an external act resulting from some internal drive. It is, therefore, a mixed reaction of physiological developments governed by heredity arousing sexual instinct.

### II. SECONDARY SEXUAL CHARACTERS & SERVING ABILITY

In animals secondary sexual characters mean, characters like the development of the body, ferocious temperament,

masculine build, pronounced sex desire (sex libido), large head, big massive neck, well developed forepart including the withers, well developed dewlap and brisket, slim hind portion (straight and broad back), capacious and tight barrel, strong and squarely placed quarters and masculine voice. The tusks of the boar, the comb and spurs of the rooster, the well developed crest and the general massiveness of head and shoulder of the bull and stallion are some of the specific secondary sexual characters. A real type of the masculine bull is the Bison. For Indian cattle (*Bos indicus*) the large well developed and tight hump with dark colour in the neck and shoulder region is a special secondary sexual character. Bulls with such highly developed sexual characteristics obviously have very high sex libido (sex-desire) and are in general very good in serving ability. Bulls which have poor sex libido show little interest in sex, timidity in action and take more reaction time for service. If the various breeds are compared, large differences in the development of secondary sexual characters will be observed. Holstein Friesian bulls are as a rule, very masculine with pronounced development of secondary sexual characters and majority have an excellent serving ability.

### III. HORMONAL CONTROL OF SECONDARY SEXUAL CHARACTERS AND PUBERTY

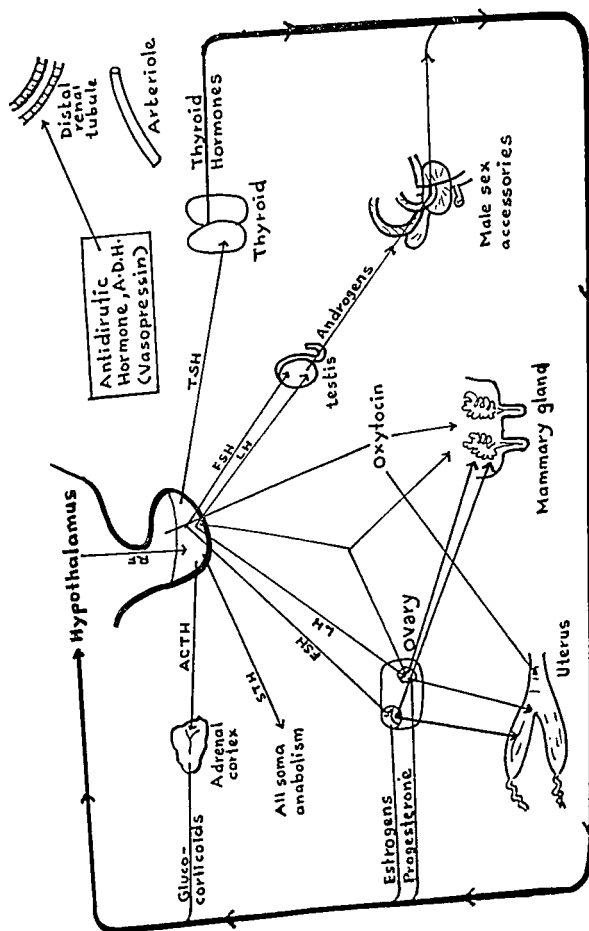
Normally growth and development of the reproductive tract and gonads is a gradual process and is comparatively slow before puberty. These organs do not exhibit any functional activity until puberty is reached. The progressive development of the reproductive organs is dependant on age and body weight and to a certain extent on heredity.

From the observations of Gilmore *et al*, (1941) and Willie (1944) in cows it seems quite logical that in males too the development and maturation of the reproductive tract may occur in three phases viz. the first is the maturation of the pituitary gland, the second is the maturation of gonads and the third is the maturation of the reproductive organs. With the consequence of pituitary growth, its hormonal influence on body growth and gonadal activity, there is a rapid increase in the gonadal weight resulting into the growth, development and functional activity of the reproductive organs. Therefore, it is quite apparent that puberty is controlled by the interaction of the anterior pituitary and gonads. With the optimum gain in weight puberty sets in. It evidently depends on a change in the balance between the output of growth hormones and gonadotrophins by the anterior pituitary (Hafez, 1952).

The exact mechanism through which the endocrine factors regulate the onset of puberty in males is still not clearly known. However, it is now clear that FSH and ICSH are available in the pre-pubertal males, their gonads can also respond to the gonadotrophins and the administration of exogenous testosterone

will suppress the release of FSH and ICSH suggesting that the negative feedback mechanism for regulation of hormone release is functional. Hafez (1974) holds the view that upto the onset of puberty the sensitivity of the mechanism is very high so that the gonadotropin level, after a burst during differentiation of the negative steroid feed back, remains very low and is insufficient to stimulate spermatogenesis. According to him the onset of puberty may be explained as decrease of brain steroid receptor sensitivity with an increase in gonadotropin secretion and subsequently with the activation of spermatogenesis. The experiment of Wurtman *et al*, (1968) producing precocious puberty after pinealectomy of young animals shows that the pineal gland inhibits onset of puberty and may lead one to postulate that pineal secretion (melatonin) can modulate the sensitivity of brain steroid receptors.

The pituitary gonadal relationship controlling the puberty is explained in Fig. 122. The secretion of FSH (follicle stimulating hormone) and STH (somatotrophic hormone) from the anterior pituitary is continued from the prenatal life but the FSH is not released from the anterior pituitary in functional quantity until later during the post-natal life. With the result, the development and growth of the body and genital organ, during this period remains under the active influence of STH. The secretion of ICSH (interstitial cell stimulating hormone) however takes place sometime after birth. Although the male gonads produce testosterone in foetal stage, the quantity produced at birth and thereafter is very low and not measurable. As the physical growth is attained there is gradual reduction in



Interrelationship between hypothalamus, pituitary gland, gonads and other target organs.

Fig. 122

the level of STH hormone which stimulates the release of FSH and ICSH from the anterior pituitary. It seems apparent that the testes respond to the action of ICSH earlier as a result of which the interstitial cells (cells of Leydig) start secreting the male sex hormone, 'Testosterone' and that is why androgen is secreted before the sperms are produced (Lindner and Mann, 1960). Since the body still remains under the action of STH, the physical growth and development still goes on and during this period the available testosterone is utilised for the growth and development of genital organ especially the accessory sex organs. When the body further reaches an optimal extent of physical growth, the growth rate then remains steady probably due to the drop in the level of STH. The testosterone at this stage does its main job i.e. it stimulates the secondary sex characters and sex libido to appear. Simultaneously, it also helps FSH to stimulate spermatogenesis to occur.

The development of secondary sexual characters depends upon secretion of ICSH from anterior pituitary. High level of ICSH will influence the interstitial cells of testes to produce testosterone. Feminine type of bulls will obviously have less ICSH.

In India, the cow bulls of milch breeds like Sindhi, Sahiwal and Gir are generally docile and usually have a loose preputial sheath while as the cow bulls of the draught breeds viz. Khillar, Ongole, Amrutmahal and Hallikar are ferocious and have tucked up preputial sheath. The secondary sexual characters are well developed in them and sex libido is excellent.

Lagerlof (1951) considers that most of the sexual weakness e.g. poor serving

ability is due to hereditary factors. He further states that males with high endocrine constitution will do well even under adverse conditions while as bulls with poor endocrine constitution may fail even in good environment (Fig. 123). Between these two extremes, there may be a number of intermediate types of bulls which are more or less sensitive to influences of climatic, nutritional and managerial conditions.

#### IV. PUBERTY AND NERVOUS SYSTEM

Some observations in laboratory animals and man reveal that nervous system may be related to the onset of puberty. In rats hypothalamic lesions produced experimentally hasten the onset of puberty. A number of pathological conditions of hypothalamus have been observed to cause precocious puberty in children. Although very little work is done in farm animals, it is possible that nervous system may also be related to the onset of puberty.

#### V. SEXUAL MATURITY

Puberty does not signify full or normal reproductive capacity (sexual maturity) which develops later. Certain developmental changes, therefore, are noticed in the genital organ, sex libido, and seminal picture of the male till some period after puberty where full potency for optimum fertility is obtained. Observations have revealed that the ejaculate volume and sperm concentration had a clearly established positive relationship with the age of the bull (Hultnas, 1959; Almquist and Cunningham, 1967; Christian and Wolf, 1963; Abdel Raouf, 1965). Abbel Raouf (1965) stated that the number of spermatozoa with proximal protoplasmic droplets decrease in bulls from 58% at

**STRONG ENDOCRINE  
CONSTITUTION**

**WEAK ENDOCRINE  
CONSTITUTION**

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++		++		+	++		
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	++ +			+			

- 1) central nervous system (Hypothalamus).
- 2) neuro-endocrine system.

Endocrine system.

3) capacity of the tissues specially the sex organs to respond to stimulation from the neuro-hormonal system.

4) unknown factor.

Fig 123

from the neuro-hormonal system.  
 1) unknown factor.

Fig. 123



9 months to 0.2% at 12 months of age. He further observed that bulls aged one year or over lie within the normal range (2% to 3%). Lagerlof and Carlquist (1961) found the reduction in protoplasmic droplets and pathological sperm in the boar from 150 to 200 days of age. Baker and Van Demark (1952) observed that the average age of first ejaculation in bulls was a few weeks later than the average age of first mounting. They attributed that during the period from first sexual interest to first ejaculation the penis grows and develops rapidly the glans penis is freed from adhesions with the prepuce and undergoes its final changes in shape and size. In the later part of this period protrusion of the penis from the prepuce becomes possible for successful service.

## VI. AGE AT PUBERTY & SEXUAL MATURITY IN CATTLE

The bull calves of exotic breeds usually reach puberty by about 10 to 12 months of age (Hafez 1974). Spermatozoa were observed to appear first at 224 days (Phillips and Andrews 1936). Baker *et al.* (1955) observed the arousal of sex desire in Holstein bulls at an age of 29 weeks.

It is generally observed that Zebu and buffalo bulls produce spermatozoa first at the age of 12 to 16 months but seldom donate semen at this age. Zebu calves usually attain puberty at 16-18 months of age. Buffalo bull calves take 1 to 6 months more to attain puberty as compared to zebu calves (Tomar 1970). A Hariana bull can be prepared to donate semen at 18 months of age if it is maintained at high plane of nutrition. Similarly, a buffalo bull in that case may donate semen at 22 months of age. Satisfactory donation of semen from Zebu bulls and buffalo bulls at 24

months and 28 months respectively may be possible if they are provided standard nutrition during their growth period. On an average the age at maturity for Zebu and buffalo bulls for ejaculating semen is 2½ and 3 years of age respectively (Tomar 1970). Kodagali (1974) on a study of 26 buffalo bulls of Surti breed observed that the first collection was obtained at an average age of  $750.03 \pm 52.36$  days with an average body weight of  $271.83 \pm 9.84$  kg. He further observed that optimum semen quantity was obtained from Surti buffalo bulls at an age of  $814.84 \pm 32.26$  days with an average body weight of  $308.22 \pm 8.64$  kg.

## Sheep

Rams generally reach puberty between 100 to 150 days or even later. Ejaculation with live sperm resulting in fertility may be obtained as early as 112 to 185 days with testicular weights being 65 gm or more as compared to adult weight of 200 gm in exotic breeds (Hafez 1974).

## Pig

Boars attain puberty at about 110 to 125 days of age. Although, the mounting activity in boars may be observed as early as 10 days of age but erection of penis is not observed until 4 months of age. Sequential sexual behaviour however, is noted after 5 months of age. First ejaculation may be obtained at 5 to 8 months but the semen volume and sperm concentration continue to increase upto 18 months.

## Horse

Stallions may reach puberty at 24 months of age when they show intense sexual desire towards mares in oestrus.

Spermatozoa are first produced in testis at about one year of age and in ejaculate they may appear at 13 months of age, although ejaculation may be obtained in artificial vagina 4 to 14 weeks before sperm appearance (Skinner and Bowen, 1968). In general practice it is not advisable to use stallions until they are 3 to 4 years of age.

The average age at puberty in males in domestic animals is as under:

Cattle	9 to 18 months	European breeds.
"	18 to 24 months	Zebu
Buffalo	24 to 30 months	
Horse	12 to 24 months	
Sheep and Goats	4 to 8 months	
Boar	3 to 6 months	
Dogs	5 to 12 months	
Cats	6 to 12 months	

Attainment of puberty does not qualify the male for service since sexual maturity is reached sometime later. Because of the individual differences in the libido, ability to produce large number of normal spermatozoa and sperm reserves, a sexually immature male should not be used even sparingly at puberty.

## VII. FACTORS AFFECTING THE AGE AT PUBERTY AND SEXUAL MATURITY

The following factors may influence sexual maturity

### Genetic

Like other characters, puberty is also a result of interaction of heredity and environment. Genetic factors affecting the age of puberty and sexual maturity are reflected by the variations in the ages due to species, breed, strain and individuals. Zebu cattle in general reach puberty from 6 to 12 months later than the European breeds. In-breeding delays the puberty while crossbreeding causes it to occur at younger age which indicates that this character is due to non-additive nature of genes.

Banc (1954) experimenting on identical twins had shown that different levels of nutrition did not have any appreciable influence on the libido and service behaviour of bulls at pubertal age and in adulthood. His work clearly indicates the role of genetic factors in male reproduction.

### Nutrition

tal effect and delays puberty probably by suppressing the secretion of gonadotrophins. Experimenting on identical twins of cattle Mann and Rowson (1956) observed the appearance of fructose and citric acid in seminal fluid delayed by 4 months and the appearance of first sperm delayed by one month in the twin partner which was raised on underfeeding.

The physiological mechanism delaying onset of puberty due to obesity is not understood. It may be possible that the fat accumulating around the testes and other reproductive tract may interfere in the secretion of reproductive hormones or it may absorb the hormone from the blood stream so that sufficient quantity of hormone is not available for the proper action. Deakin (1943) held the view that the accumulation of fats around the testes may form an insulation over the testicular tissue as a result of which the spermatogenesis may be impaired.

However this is true, that due to obesity the male animals become sluggish and lazy as a result of which they are not able to perform courtship due to physical inability although they may have sexual interest in the female.

Deficiencies in some nutritional components viz. vitamin A, phosphorus, calcium etc. may delay the onset of puberty due to slow rate of growth. Though, no significant beneficial effects have been observed by feeding grains on the sexual maturity in bull calves, the importance of grains in the bull ration for higher growth rate and earlier sexual maturity cannot be ruled out (Pakenas, 1961; Monciardini, 1964). It is generally viewed that there is no superiority of animal protein over plant protein on the sexual performances.

Deficiency of vitamin A in the diet of the growing bull calves before reaching puberty has proved to have disastrous effect. Hodgson *et al*, (1945) observed absence of sex libido, degeneration of seminiferous epithelium and formation of pituitary cysts in such bull calves. Supplementation of vitamin A did not restore their reproductive functioning. Similar effects of vitamin A deficiency was also recorded by Erb *et al*, (1947) in the bull calves which ultimately delayed the puberty. Tomar (1970) reported a higher growth rate and earlier sexual maturity in Haryana and Murrah buffalo bulls fed on standard rations.

### Body weight

Progressive weight gains in the growing male indicates the growth rate. The body growth is directly influenced by the level of nutrition and as such the weight of the male animal is generally considered as a good criterion for recommendation of the male for service. Average live weight of the bulls should also be considered when they are taken up for training for semen collection or natural service. According to Tomar (1970) the Zebu bulls on an average should weigh over 230 kg. at an average age of 16 to 18 months when they are first put to service and they should weigh not less than 300 kg. at 2 years of age when they start giving satisfactory semen. Tomar (1970) further considers that buffalo bulls at sexual maturity should weigh 10 to 20 kg. more than the cow bulls.

### Climate

Climatic factors influence the age at puberty. In sheep, pig and cattle season has considerable influence on the onset of puberty.

Seasonal variations in the quality of semen however, was observed in adult males of different species of farm animals. (Mukherjee and Bhattacharya, 1952; Shukla and Bhattacharya, 1952a, 1952b).

## Hormones

Pituitary gonadotropic function sets in very early in fetal life. Negative feedback mechanism of gonadotropin regulation is initiated by the first secretion of testosterone which occurs in fetal life. Experimental evidences have shown that the pituitary gland contains adequate quantity of gonadotropins during prepubertal period but these fail to be released in general circulation. Spermatogenesis and steroidogenesis of the testes therefore, can be stimulated by exogenous gonadotrophins a long time before puberty (Hafez, 1971).

of the individuals of the same sex delays onset of puberty (Hafez, 1971).

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# Chapter 35

## Andrological Investigation

### COW-BULLS AND BUFFALO-BULLS

A large number of bulls are in use for natural service under village conditions and at cattle breeding projects including semen collection centres. These bulls are either farm bred or purchased. Many a times, these bulls are put to use before they are thoroughly investigated for their reproductive health, sex libido, sexual behaviour and semen quality, in consequence of which bulls with lowered reproductive efficiency escape notice. As such it would be advisable to conduct detailed andrological investigations before the bulls are selected for breeding.

#### 1. Anamnesis

The following information concerning the life history of the animal is essential.

- (1) Pedigree for three previous generations.
- (2) Date of birth.
- (3) Age at puberty (age at first service).
- (4) Frequency of service/semen collections.
- (5) No. of services/collections during the last twelve months and number of pregnancies obtained.

- (6) Protection against infectious diseases.

#### 2. General Health and Locomotion

The bull should be examined for general health. He should be free from any apparent disease. The eyes, ears, mouth, nostrils, prepuce and anus should be examined for any pathological discharges. The bull should not be pot-bellied since he will be unable to raise his body and balance correctly on hind limbs during coitus. The hooves must not be over grown and locomotion should be normal. Before andrological investigation, the bull may be made to walk in order to observe its locomotion. Limbs may be carefully examined for injury to hooves, clefts, sprain, tendinitis, arthritis and fractures. In brucella infected bulls, arthritis and hygroma of the joints are common. If the bulls are lame specially in the hind limbs, service ability will be affected. The bull should have lustrous, shiny coat, with no external parasites. It will be advisable to pull few hairs from three or four different places and examine under microscope for parasites.

The bull should not have any abnormalities like malformed feet and hernia. It is important to look for such abnormal-

malities as many of these defects are transmitted.

### 3. Development of Secondary Sexual Characteristics

The bull's anterior portion near the shoulder region should be massive and well developed. In general the bull should give a trim appearance and not be of fatty type. The development of dark colour in the neck region specially in grey, white and red zebu breeds of cattle indicates sexual development. This colour starts developing nearer the age of puberty and becomes intense when the bull is fully sexually mature. This dark colour is not seen in castrated bulls.

The hump in Zebu bulls is well developed, massive and erect. Humps partly leaning on either side are not preferred by the breeders. The preputial sheath should not be loose but well tucked up and tight. The draft breed bulls — Nagauri, Haryana, Khillar, Amrit Mahal have tucked up sheath but in milch breed animals namely Red Sindhi, Sahiwal, Tharparkar, the incidence of loose preputial sheath is quite common and such bulls are lethargic and have low sex libido. Besides this, the loose preputial opening gathers dirt and contaminates semen ejaculate when discharged.

The bull should have masculine voice and he should be somewhat aggressive with masculine temperament and must not be docile like a female.

### 4. Sex Drive

The reaction time of a bull should be observed in order to assess his sexual desire. Reaction time is measured as the time taken to complete the process of

service in the presence of a female in oestrus or in anoestrus. The reaction time varies between half to two minutes with a maximum of ten minutes. Bull reluctant to mount the female even after 15 minutes of teasing, should be considered as a problem case. The penis of the bull soon gets erect and a bull with normal sex desire will mount immediately and serve. No pre-coital foreplay is observed in bulls as in stallions and dogs. Fibro-elastic penis of the bull contains less vascular tissue and hence erects rapidly. Even in non-erect state, the penis of bull is hard on palpation. Good sex desire and service behaviour cannot however be considered as guarantee for fertility.

The sex libido in bulls may be categorised as follows:

- A. Excellent — Mounting within half minute.
- B. Good — Mounting between half to one minute.
- C. Medium — Mounting between 1-2 minutes.
- D. Poor — Mounting after 2-5 minutes.
- E. Lack of libido — Not mounting at repeated attempts.

### 5. Service Behaviour

The bull's behaviour during service should be carefully observed. A normal bull with intense sexual excitement will at once mount a cow, raising his body on the hind feet and gripping firmly at the shoulder region with his fore legs. At the same time the erect protruded penis will make the searching movements to locate the vulval opening. As soon as it is done, the bull will give a forceful thrust by lifting his hind limbs raising them few inches from the

ground. Simultaneous with the forceful thrust semen will be ejaculated and deposited in the anterior portion of the vagina. At the same time peculiar wave like movements at the lumbo sacral region of the bulls are seen. After discharge of the semen the bull will quickly dismount and the penis will lose its rigidity and will be withdrawn in the preputial cavity. Sometimes after dismounting trickling of a few drops of ejaculate may be seen. If the bull is not withdrawn from the cow, he may soon regain the libido and will attempt to mount again. This is observed in case of bulls with good sex-libido.

In many collection centres it has been observed that the A.V. during collection is not held at the level of vulva to allow the bull to jump and ejaculate in the A.V. to obtain good thrust. Instead the A.V. is lowered and as a result the bull is not able to give the forceful thrust. The quality of ejaculate thus obtained may be also not high. By such erroneous methods of collection the bull's sex-libido also gets disturbed and he will become lithargic.

## 6. Serving ability

The serving ability may be evaluated by observing service behaviour at the time of semen collection with artificial vagina.

Serving behaviour is influenced by several factors like presence of people, movement and size of teaser and the type of flooring. The serving behaviour can be graded from 0.6 based on the following (Hultnas, 1959):

- (a) Libido (no interest to mount to very willing to mount).
- (b) Erection during seeking and stiffness of penis. (no erection after mounting to very good erection).

- (c) Erection during seeking — length of protruding penis. (no protrusion to abnormally long protrusion).
- (d) Manner of seeking. (no seeking movements to determined seeking movements).
- (e) Thrust. (no release of thrust even after contact with vaginal mucosa to good thrust).
- (f) Body position during seeking. (marked concavity of lumbar area to failure to grip the cow with forelegs).

## 7. Examination of the Reproductive Tract

### A. PREPUCE

Preputial cavity should be examined for its hygienic conditions. In breeding bulls the preputial tuft of hairs is often clipped and the area is cleaned of hairs. The cavity should be examined for adhesions, pathological lesions, discharges and trauma. In bulls of the milch breed namely Red Sindhi, Sahiwal, Gir and Tharparkar, the prepuce is often loose and in some bulls even the prolapse of prepuce is noticed. In such cases the preputial mucous membrane is exposed and may get easily infected (Fig. 124).



is many a times responsible for such injuries when the bulls are running with females in an enclosed area. At times the penis also gets fractured. Full protrusion of penis during erection should be looked for. It may be short and protrusion may only be one or two inches. This is because of short length and this malformation has hereditary predisposition. There may be a tumour in the penile region and the protruded penis can not be withdrawn in the prepuce (paraphimosis). The defects in retractor penis muscle may also be looked for.

### C SCROTUM

Scrotum should be inspected to detect asymmetry, scrotal raphae and skin lesions. Scrotum and scrotal contents are palpated for size, form, consistency and mobility.

### D TESTES

The shape, size and consistency and location of testes in the scrotum should be noted and compared. The testes should be easily movable in the scrotum normally. In acute orchitis the testes will be enlarged and painful on palpation and in chronic orchitis the testicles become harder. In case testicles are not reducible in scrotum, adhesions can be suspected. Only one testicle may sometimes be present (monorchid) and other held in inguinal canal or there is incomplete descent of both testicles (cryptorchidism). There may be tumour in the testes and careful palpation will reveal this condition. While palpating the testes, careful observations should be made as to the difference in size of testicles. Apparent disparity in size is indicative of hypoplasia and such a testicle is softer in consistency. Repeated examinations of semen samples reveal the defect. Each testicle and epididymis

is examined separately. The head, body and tail of the epididymis are palpated. Consistency of the testis consists of two components: firmness and springiness. Firmness varies from very firm to very soft. Springiness varies from very good to very poor. In normal sexually mature bulls the consistency is firm and springy; in young animals it is soft.

### E EPIDIDYMIS

The shape and consistency of caput corpus and cauda of the epididymis are determined by palpation. In case of inflammation these parts will be enlarged and painful to touch, as is observed in epivag. Epididymis should be palpated and cauda examined for spermiostris.

### F VAS DEFERENS

These are palpated for abnormality, if any.

### G ACCESSORY SEX GLANDS

Rectal examination of the bull is necessary for investigation of accessory sex glands. A lubricated gloved hand is inserted in the rectum. The pelvic urethra can be palpated lying on the caudal portion of the pelvic floor. Ampulla can be palpated as broadened terminal parts of vas deferens lying across the bladder and terminating at its neck. Inflammatory changes (ampullitis) can be detected on palpation. The seminal vesicles are palpated on each side of ampullae as distinct elongated lobular structures. The two lobes of seminal vesicle are palpated for size and consistency. In young bulls they are about 6.8 cm long, 2.3 cm wide and 1.2 cm thick. The consistency is soft in young animals and becomes firmer with age. The seminal vesicles are mobile in normal bull and the mobility is lost in inflammatory conditions. The

consistency and shape of vesiculæ seminalis may be ascertained to detect inflammatory changes. It has been commonly observed that if right testicle has been affected then the seminal vesicles on the same side also get involved and sometimes adhesions can be felt (semio-vesiculitis). Infection of seminal vesicles is common in bull and *Pseudomonas aeruginosa*, has been isolated by several workers. Small, under-developed, cystic or even absence of seminal vesicles may be observed in some bulls. In buffalo bulls the seminal vesicles are comparatively smaller and less lobular than in cow bulls. This accessory gland is more predisposed to disease condition than the other accessory glands or the ampulla in the bulls. The malformation of vesiculæ seminalis may be due to the defective development of Wolffian duct system during intra-uterine life.

Cowper's glands or bulbo-urethral glands cannot be palpated as they are covered with muscles. These glands seldom get diseased.

The prostate gland is palpated as a thick transverse fibrous band of tissue around the urethra at the cranial end of the pelvic urethra and just caudal to neck of the bladder and ampullae and vesiculæ seminalis. This gland also rarely gets affected except in dogs.

#### H. EXAMINATION OF SEMEN

This is of great diagnostic value in determining the quality of semen and in disturbed conditions the severity and degree of testicular pathology. The various tests for examining semen quality are as follows:

(i) *Volume*: Small volumes of semen may be ejaculated in young males in cases of incomplete ejaculation and in diseased conditions like bilateral

seminal vesiculitis. The bovine ejaculate should be more than 2 ml. at one collection.

(ii) *Colour*: The usual colour is milky or creamy, creamy white and opaque. In case of orchitis semen may be of brownish colour. Some bulls may produce light yellow coloured semen but this is normal. In some cases yellowish green colour may be seen due to the presence of *Ps. aeruginosa*. A brick red to pink colour of the semen is due to the presence of blood in the genital tract.

(iii) *pH*: In bulls and rams the pH of semen is about 6.7. In pathological or inflammatory conditions affecting testis, epididymis or seminal vesicles, the pH of the semen is usually 7.0 or above.

(iv) *Concentration*: The normal sperm concentration in the bull varies from 300,000 to 2,000,000 per cu. mm with an average of 800,000. Concentration of spermatozoa below 600,000 should be looked upon with suspicion. In testicular hypoplasia, the sperm concentration is usually less than 75,000 per cu. mm.

(v) *Motility*: Most fertile bulls have 60-90 per cent of motile spermatozoa in their ejaculates. Motility below 50 per cent is often associated with pathological conditions of testis and epididymis. Sperms with defects of mid piece and tail show the lowest motility. Epididymal disturbances affect the motility of sperm.

(vi) *Live and dead spermatozoa*: An average of 80 per cent live spermatozoa is usually observed in normal semen samples. A minimum of 60 per cent is acceptable.

(vii) *Sperm morphology*: The morphology of sperm head is best studied

in preparations stained with William's stain and that of mid piece and tail studied in wet preparations with buffered formal saline

There is an average of 20 to 25 per cent abnormal head forms in semen from bulls of good fertility. A total content of 18 per cent of abnormal sperm is considered to be within the normal range. The incidence of proximal protoplasmic droplets should not be more than 2 per cent to 5 per cent in normal bulls. Higher frequency indicates disturbances like testicular degeneration and hypoplasia.

Certain morphological defects probably of hereditary origin are reported to produce infertility in bulls. Special attention has to be paid for the morphological defects of spermatozoa as described in earlier chapters.

## 8 Idiosyncracies and Vices

Some bulls will mount on teasers of particular colour. If the semen collector wears bright or white dress his presence is not liked by some bulls. The sexlibido is disturbed if timings of semen collection and place are changed in some instances. Certain bulls react to male teasers only. Details of such psychological aberrations if encountered should be carefully noted.

Bulls may have different types of vices such as fighting and masturbation. The habit of masturbation usually develops in young bulls when they are tied in bull pens adjacent to the cow byres and also due to high protein feeds. They do not get an opportunity to serve the females but can smell and see them. This habit in fact can be described as due to intense sexual instinct and may not be considered as harmful unless the masturbation is very frequent.

The vices develop mostly due to defective rearing during younger age.

## 9 Feeds and management

The details of the ration schedule and the amount of ration fed and timings may be obtained with a view to study imbalance in feeds which might lower the reproductive efficiency. The stalls or bull pens where the bulls are located, the different managerial routine including exercise and watering should also be looked into.

## 10 Fertility

The real index of the bull's reproductive efficiency can be ascertained by studying the rate of conception. Two years' breeding records should be studied if available. At least data pertaining to the females served by the bulls artificially or naturally during six months previous to investigation should be obtained to determine the conception rate of the bull.

In newly acquired bulls whose fertility records are not available, it will not be possible to determine the conception rate. In such cases it will be advisable to examine the heifers and cows recently served by him for determining early pregnancy.

The various observations described above can be recorded in the standardised proforma.

## 11. Venereal infections

It is necessary to conduct the tests for the detection of vibriosis, trichomoniasis and brucellosis, when the herd records indicate lowered fertility in bulls.

## STALLION

The general principles and methods of investigation of stallion are the same.

as detailed for the bull. However, certain anatomical and physiological peculiarities in stallion will have to be borne in mind while examining a stallion.

#### (i) Reproductive organs

Extra care and restraint of the stallion is necessary before attempting examination of genitals. The scrotum has a thin skin and the testes lie in almost horizontal plane with the vas deferens lying dorso-medial. On traction, the testes assume almost vertical position. On palpation the organs are more flaccid and less resilient than in bulls. The head of epididymis is cranial and distinctly marked while the tail is caudal. The testes and epididymis should be freely moveable in the scrotal sac and should be free of gritty or granulomatous lesions.

Penis of a stallion contains more of cavernous tissue and is therefore capable of increasing double in length and thickness at the time of erection. Examination of penis can be done by gentle traction of the organ from prepuce with a gloved lubricated hand. It is advantageous to tranquilize the stallion before this. The urethra can be felt as a compressible tube on the ventral aspect of the cylindrical penis and its palpation at the time of service into an artificial vagina can give one the idea of thrust of semen at ejaculation.

Rectal examination of the pelvic organs of stallion requires special care and restraint of the animal. The tail should be bandaged in order to prevent the intruding of hair into the rectum. Frequent lubrication of the gloved hand and gentle manoeuvres have to be practised. The seminal vesicles are smooth on palpation in contrast to that of bulls. Tracing of the vas-deferens

upto the inguinal ring, in order to locate the latter is of special significance in the examination of stallions with retained testes.

#### (ii) Service Behaviour

Anatomical peculiarity of having a typical musculo-vascular penis with no sigmoid flexure necessitates certain amount of foreplay in the service behaviour of stallions. Efficient intromission is possible only on complete erection for which courtship paves the way. At the sight of mare the stallion produces characteristic neighing and shows signs of "excitability". It smells the external genitalia and groin of the mare and extends its neck with an up-curved upper lip. Smelling is followed by pinching of the croup region of the mare by more or less firm grasp of the mare's skin in between the teeth.

The whole process of male behaviour could be classed as three phases — (1) courtship, erection and mounting, (2) intromission and (3) ejaculation. Erection begins gradually while the stallion is some distance from the mare and completes towards the end of courtship. At full erection the free part of penis will be 12 to 20 inches long on the dorsal side. After erection the animal mounts the mare. The stallion leans over the mare after gripping it at shoulders with forelimbs. Usually it bites the mane of the mare with its teeth. Sometimes more than one mount may be performed at one copulation (ranging from 1 to 4). At each mount the stallion performs several pelvic oscillations and then dismounts. After full erection, helped by pelvic oscillations, intromission takes place. On an average after 13 seconds of the few copulatory movements first ejaculation occurs and the whole process of ejacula-

tion is complete in 10 seconds with 10 pulsations of the urethra on an average. Cessation of pelvic oscillation is the sign of beginning of ejaculation. The tail is also moved up and down in a characteristic fashion during ejaculation. Most stallions, exhibit fast respiration, dropping of the head and relaxation of whole body during ejaculation. The stallion then dismounts the mare with a flaccid, loose penis which is soon withdrawn into the prepuce. Young stallions show erection and mounting at the age of 6-8 months, but maturity is attained by 12-18 months only. Though sex desire is manifested throughout the year, it is higher in spring than in autumn or winter. With advancing age there is however some reduction in sex drive.

Certain aberrations are sometimes noticed in the service behaviour of stallions. Inability to ejaculate in spite of erection is sometimes noticed especially in young ones and over-used ones in the peak of breeding season. Inability to serve A. V. is sometimes noticed. Many a times this is due to faulty application of A. V. It is worthwhile to mention, in this context, that pressure of the A. V. on penis is the important factor in addition to temperature of A. V. to be taken care of in collection of semen from stallion. Temporary lack of sexual expression is sometimes observed in young stallions and in older ones isolated in box stalls without exercise. Incomplete intromission and lack of pelvic oscillations at times observed may be due to hereditary predisposition. Sexual inhibition may be accompanied by excessive irritability as reflected by excessive biting of the mare on approach and on mounting. Masturbation with or without ejaculation is sometimes observed as vice in stallions. Other vices such as stall-walking, weaving, cribbing and self-

biting should also be looked into in examination of a stallion.

## RAM AND BUCK

The external and internal genitalia of ram and buck are similar to that of bull except for differences in size and minor details. The penis of ram and buck is characterized by a urethral process extending 4-5 cm. beyond the glans penis. The service behaviour is almost the same as in the bull. However, courtship characterised by sniffing of the external genitalia, smelling of secretions from genitalia and peculiar stance with extended neck exposing the teeth with up-curved lip is more pronounced. Erection, mounting, intromission and ejaculation are quick as in bulls. In artificial semen collection, the warmth and air pressure in A.V. is most important as stimulus for ejaculation. Testes are examined for the normal size, texture or otherwise, for adhesions to the scrotum, varicocele (Fig. 124a), orchitis etc. All parts of epididymis and vas deferens to a certain length can also be palpated as in bulls. As it is not possible to investigate the animals per rectum, palpation of internal accessory genital organs is not possible.

## BOAR

The scrotum bearing the testicles is placed more caudally and ventrally to the ischiatic arch. The long axis of testis within the pouch is almost vertical with its free border caudally. The tail of epididymis is dorsal and quite large. The penis is fibro-elastic in nature and with a sigmoid flexure which is pre-scrotal. During erection about 20-35 cm. of the organ is protruded free of prepuce. The cranial end is devoid of a glans, but ends up in counter clock-wise spiral twisting. The preputial orifice is narrow and on

its dorsum is a preputial diverticulum which when filled with urine and smegma, resembles an umbilical hernia. This region is provided with sebaceous glands, the secretion of which gives the characteristic 'boar odour'. The accessory organs resemble those of bull except for the large size of seminal vesicles. As the size of animal is small it is not possible to palpate the internal accessory sex organs.

One common vice developed by boar is masturbation by inserting the penis into the preputial diverticulum and ejaculating into it. Once this has developed it has to be corrected by surgical removal of the diverticulum. This point may be noted while examining boars. Also rectal copulation (pederasty) is common in young boars in A.I. studs. In the examination of boars, the increased proneness of this species to cryptorchidism and pseudohermaphroditism should be taken into account and careful search made in the pedigree and to eliminate these developmental defects.

The testis may be palpated for orchitis adhesions to the scrotum and the

is slow and therefore foreplay is essential. The coitus in dog takes more time ranging from 5 to 25 minutes.

## PROFORMA FOR ANDROLOGICAL INVESTIGATION OF SIRES

(Lagerlof, 1969)

### 1. Signalment of the bull

- (a) Breed
- (b) Age
- (c) Colour
- (d) Type

### 2. History

### 3. General Investigation

- (a) Habitus (Posture, flesh condition and temperament)
- (b) Visible mucus membrane
- (c) Palpable Lymph Glands
- (d) Skin (Hair coat)
- (e) Temperature

### 4. Investigation of the different systems

(a)

- (iii) Ruminal movements
- (iv) Reticular movements
- (v) Faeces (amount, consistency, colour, smell, presence of long straws, parasites)
- (vi) Examination of the liver

**(d) EXAMINATION OF THE URINARY SYSTEM**

- (a) Palpation of the left kidney per rectum and percussion of the right one.
- (b) Examination of urine (specific gravity, volume, albumin and sugar).

**5. Special Investigation of the Genital Organs and Semen and Sexual Behaviour**

**I. INVESTIGATION OF THE TESTICLE**

- (a) Inspection of the scrotal sac and the skin of the sac
- (b) Palpation of testicles inside scrotal sac, especially concerning movement of the testicle
- (c) Special Investigation of the testicle:
  - (i) Size and symmetry (length and breadth)
  - (ii) Consistency (firm or hard)
  - (iii) Position of testicles — Normal or crossed (Fig. 125)
  - (iv) Palpation of Epididymis (head, corpus and tail)
- (d) Palpation of prepuce, penis and urethra (hypospadias or epispadias, Fig. 126)
- (e) Investigation of accessory sexual glands (seminal vesicles for size and consistency, lobulation; ampullae for symmetry and palpable part of prostate gland)

**II. INVESTIGATION OF THE SERVING BEHAVIOUR**

- (a) Libido
- (b) Erection
- (c) Mounting the cow
- (d) Penile movements to locate the vulva
- (e) Actual thrust
- (f) Ejaculation
- (g) Dismounting (*Observe each and every movement during every service of the bull.*)

**III. SEMEN INVESTIGATION**

- (a) Estimation of the ejaculated volume and semen's macroscopic appearance
- (b) Estimation of mass activity and motility
- (c) Estimation of pH and catalase activity
- (d) Morphologic investigation (examine carefully for the presence of any cells other than sperms e.g. leucocytes, giant cells or other cells)
- (e) Bacteriologic investigation of preputial washings.

**6. Serological Investigation:**  
e.g. Brucellosis.

**7. Short summary of findings**

- (1) History
- (2) Sexual behaviour
- (3) Clinical findings
- (4) Semen examination

**8. Comments on the findings**

**9. Diagnosis**

**10. Prognosis**

**11. Recommendations**

**ANNEXURE**  
**INVESTIGATION OF BULLS**

Name of the owner:

Date:

Name of the Herd/Village:

Bull/ Buffalo-bull .....

Species and Breed:

No. and/or name of the animal:

(Cross bred level 50%, 62.5%, 75%).

## HISTORY

Date of birth and/or age:

**Pedigree:**

Hereditary manifestations of abnormalities in progenies (if any):

Age when first allowed to serve:

Previous service behaviour (if known):

Record of previous services and results  
(if known):

Month	No. of Services	Result	Month	No. of Services	Results
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**Fertility rate:**

Dates of last 12 services:

Body Weight (Actual):

(Calculated):

General condition:

Feeding practices and tasks of management during growth and adult periods:



## INVESTIGATION

General health

Sexual behaviour (mention also if the cows are tied up, moved or free during service):

## IN NATURAL/A. I. SERVICE

- 
- (a) Libido No, weak, medium, good.  
Interest shown in the female
- (b) Erection  
Before mounting  
(Penis in the prepuce  
or out of the prepuce)
- (c) Penile movements  
(reaching or not  
reaching vulva)
- (d) Thrust  
(forceful and deep  
or weak and short)
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## - Semen Studies

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Date	Total Volume	Colour	Mass activity	Initial Motility	Density	Remarks (mention also about leucocytes and other cells)
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Ejaculate I

,, II

Secondary sexual characteristics:

-- Well developed/Developed/Poor

Clinical investigation of sexual organs:

Preputial sheath

Tight/medium/pendulous

Penis

Testes

Epididymis

Seminal vesicles

Ampullae

Remarks:

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# Chapter 36

## Infertility in the Male Domesticated Animals

Infertility or sterility is probably as common in the male as in the female. It is usually said that bull is half the herd. In spite of recognising the role of bull in improving the breed, male infertility has received lesser attention. Large scale adoption of A.I. has permitted extensive utilization of bulls and some bulls are known to have sired 100 000 to 200 000 cows in their life time. In this context studies on male infertility have assumed greater importance than ever before. The causative factors for male infertility as classified by Lagerlof (1938) are as per Table No. 11.

Table 44  
CAUSES OF INFERTILITY IN MALE ANIMALS

### A CONGENITAL, USUALLY HEREDITARY CAUSES

- 1 Morphological defects. Malformations such as
  - (a) Testicular hypoplasia
  - (b) Segmental aplasia of mesonephric ducts
  - (c) Spermiostasis
  - (d) Cryptorchidism
  - (e) Hermaphroditism
- 2 Congenital disturbances or spermiocytogenesis and sperm morphology

- (a) Stickiness
  - (b) Chromosomal aberrations
  - (c) Acrosomal defects etc
- 3 Predisposition for functional disturbances
  - (a) Weak libido
  - (b) Disturbances of the serving ability
  - (c) Disturbances of ejaculatory mechanism
  - (d) Disturbances of spermatogenesis

### B ACQUIRED CAUSES

- 1 Somatic diseases (Foot and mouth, Brucellosis, lymphadenitis, traumatic peritonitis, intoxications, metabolic and deficiency diseases)
- 2 Infections of the sexual organs
  - a Coital infections
  - b Non specific infections
- 3 Local damage to the testicles from heat, trauma, insects, etc
- 4 Nutritional causes (over and under feeding, deficiencies in minerals, vitamins or trace elements)
- 5 Management factors (Change of environment, inconvenient place for service, unsuitable handling)

### C. COMBINATIONS OF A AND B

Further the forms of infertility can be divided into 3 main categories as described by Lagerlof (1938).

- I. Reduced to complete lack of sexual desire (sex drive or libido).
- II. Inability to copulate (impotentia coeundi).
- III. Inability or reduced ability to fertilize (impotentia generandi).

#### I. Reduced to complete lack of sexual desire

This condition is characterised by sluggishness, delay or total lack of sexual desire. Studies on identical twin bulls by Bane (1954) have revealed that sex drive is largely determined by genetic factors though environment may play an important role in modifying it. The intensity of sex drive varies from breed to breed. Among the Indian bulls the draught breeds (Hallikar, Amritmahal and Khillar) are reported to have stronger sex drive while as in the milch breeds (Sindhi, Sahiwal and Gir) a number of bulls are observed to have

proper training may also lead to poor sex drive.

(c) *Psychic factors*: They play an important role in altering the sex drive of males of low genetic make-up. Injuries that may have been inflicted at the time of natural mating or during semen collection are usually remembered by the bulls and these factors may inhibit the sex drive. Similarly harsh and abusive handling of bulls at the time of collection and improper restraint of the dummy may also depress sex drive. The colour of the apparel put on by the semen collector is known to influence the sex drive in some cases. Size and colour of the dummy are also observed to influence the sex drive.

(d) *Hormonal deficiency*: Deficiency of hormones like thyroxine, testosterone and gonadotropins may be involved in causing decreased sexual desire.

(e) *Systemic diseases*: The sexual desire is usually reduced or depressed by chronic or acute debilitating diseases viz. pneumonia, enteritis, tuberculosis, actinomycosis, progressive pit necrosis and severe mange.

Before resorting to any treatment it is necessary to obtain the breeding history, carry out physical examination and observe the serving ability of the male. Special attention should be paid to the feeding schedule, exercise, frequency of service and size of the teaser animal. Lagerlof (1951) emphasized that males having strong endocrine constitution alone should be selected for breeding purposes.

## II. Inability to copulate (Impotentia coeundi)

In this condition, the bull may have normal or slightly decreased libido but it is unable to copulate or complete the coitus. A critical observation of the male during service is necessary to assess the serving ability. Inability to copulate may be caused due to the following conditions.

### (a) INJURIES TO THE JOINT, MUSCLE, BONE OR TENDON

Inflammation of the stiffl joint (Gonitis) is one of the condition commonly met with, which prevents copulation in bulls. Dislocation of the hip joint may also cause inability to copulate. The lesions of hip joint are not so frequently encountered in bulls compared to dogs, stallions and boars. Males with lesions of hip or stiffl joint exhibit typical severe lameness which is characterised by a short stride by the affected limb. Affections of tarsus or fetlock and over-grown claws or hooves may also interfere with copulation. Bulls affected with Foot and Mouth disease and having lesions in inter-digital space are unable to perform service though they have normal libido. The capacity to perform service is restored after the healing of wounds.

Holst (1919) observed that chronic swine erysipelas causes joint lesions.

Lagerlof (1938) stated that inflammation of iliosacral joint is relatively a common cause of service inability particularly in young bulls. Bane (1954) demonstrated that lesions affecting the ilio-sacral joint and characterised by peri-articular ossification and syn-ostosis are physiological phenomena. He did not observe any skeletal changes in or around ilio-sacral joint in infertile SRB bulls below three years of age. He observed slight peri-articular ossification in two bulls aged four years and these changes increased with age. The ossification did not indicate any pathological process. Exostosis and healed or partially healed fractures of the lumbar and last few thoracic vertebrae of 7 to 14 year old bulls were recorded by McEntee and Roberts (1954). They observed stiffness and painful gait, spinal rigidity, pain on pressure over the lumbar vertebrae in bulls, dogs and boars which were unable to serve. In acute cases there was a slight to marked paresis characterised by swaying and unsteady gait of the hind limbs.

Bulls with *spondyle arthrosis* are unable to complete the coitus as they mount the cow too far caudally which permits the extension of penis only for a short distance (Bane and Hanson, 1962).

Bulls with *spondylosis* involving intervertebral discs are unable to perform service due to stiffness of the back.

Spastic syndrome characterised by prolonged spasm of the muscles of the hindlimbs, lumbar and other regions also prevents copulation. This has been observed in Holstein, Jersey, Ayrshire and Charollais breeds. This condition is known to be hereditary and usually affects older bulls.

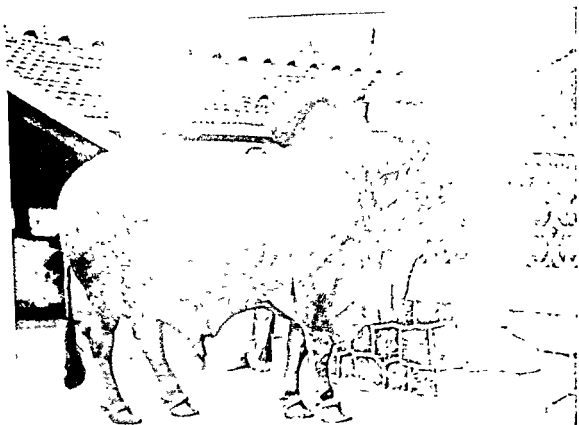


Fig. 124.  
Pendulous sheath in a Gir bull with  
balanoposthitis.



Fig. 127 Adhesions obliterating preputial orifice in a bull



Fig 125 Crossed testicles in a bull



Fig 128 Phimosis in a bull

## Treatment

For all the above conditions treatment consists of sexual rest, restriction of movements and providing proper footing. As a supportive treatment balanced feeding, application of counter irritants and massage are indicated.

### (b) AFFECTIONS OF THE PENIS AND PREPUCE

Congenitally short penis and retractor penis muscles are considered to be inherited defects. Serving ability is affected to a varying extent. Failure of normal separation of the penis and prepuce also affects the serving ability.

Deviations of the penis either ventral, lateral or of cork-screw type are also known to cause inability to serve. The deviation of penis may also be caused by the persistence of ventral frenulum. A number of such conditions have been recorded in European breeds. Very little information is available on such conditions in Indian cattle. In some bulls, the penis protrudes normally and intromission cannot take place due to defective engorgement of the penis. Deshpande *et al.* (1967) reported a case of impotentia coeundi in a buffalo bull. Rupture or broken penis with secondary haematoma is most commonly observed in young bulls with strong sex drive. The injury usually occurs at the time of coitus when there is quick bending of the erected penis against the escutcheon of the cow during thrust. Later the bull is reluctant to perform service. According to Bane (1965) the site of rupture or fracture of penis is just behind the attachment of retractor penis muscle. The symptoms of rupture of penis are arching of the back, short strides and soft and fluctuating swelling varying in size, develops cranial to the scrotum. Prolapse of prepuce may accompany the oedema. The haematoma

later on develops into a hard mass. It is painful but not hot. The blood clot may lead to adhesions. Such bulls would naturally become unfit for breeding purposes.

**Balanoposthitis:** Inflammation of the glans is called balanitis and that of prepuce is called posthitis. Often they are involved together because of their close anatomical relationship. In balanoposthitis the copulation is usually prevented due to adhesions between the penis and prepuce or to the surrounding tissues (Fig. 127).

This condition is usually caused by a wide variety of organisms including *M. tuberculosis* and viruses like IBR — IPV group. Non-infectious balanoposthitis in rams has been reported in Australia. The incidence of this condition in boars is rare.

Prognosis depends upon the severity of trauma or the infection. Prognosis is good in mild cases and guarded in chronic cases with adhesions between penis and prepuce.

Rollinson and Haq (1948) found profuse growth of *Absidia* mould in the preputial washings of a bull which was a slow breeder, having masturbating vice and had preputial catarrh. Williams (1943) stated that tuberculosis of sheath, prepuce, penis and penile lymphglands are characterised by enlarged granulomatous bleeding lesions of the glans penis, adhesions of the penis, prepuce and sigmoid flexure resulting in phimosis. He found abscesses in the enlarged penile lymph glands.

### Treatment

In mild cases of balanoposthitis douching the prepuce with aqueous or oily antiseptics or antibiotics is indicated. Sexual rest is essential.



*Phimosis or Stenosis of Preputial orifice.* In this condition the normal protrusion of penis is prevented and thereby the male is unable to perform the coitus. It is usually acquired due to injuries, wounds and infections (Fig. 128).

Vander Sluis (1953) observed 38 cases of inability to protrude the penis, out of 828 bulls examined. He further observed that 10 bulls had acquired the condition at an older age after having normally mated previously and in 28 bulls it was noticed at a younger age.

The symptoms in bulls vary from inability to protrude the penis out of the sheaths to being able to extend it about 10 to 15 cm. but not far enough to contact the cow. These bulls exhibit good sexual desire. No evident physical defects, adhesions or strictures in the prepuce are noticed.

Holst (1949) observed cases of inability to protrude the penis in the boar and attributed the cause to be hereditary. He described that the affected boars exhibited sexual desire and mounted the sows but were unable to protrude penis beyond 2.5 cm. or so out of the prepuce which remained pendulous. Intromission was therefore not possible.

Richter (1919) recorded arrested development of sigmoid flexure in goats resulting in inability to protrude the penis. Similar observations have been made by Roberts (1956) in three Guernsey, one Holstein and two Jersey bulls. Vander Sluis (1953) reported that some bulls could extend the penis normally while standing but were unable to reach vulva on mounting.

*Paraphimosis:* In this condition the animal is unable to retract the penis into the prepuce after protrusion. This

may result after resection of the retractor penis muscle in bulls. This may also occur due to paralysis of penis in spinal diseases. In horses, this condition is attributed to generalised weakness, debility, neuromuscular paralysis or following castration causing oedema of the penis and sheath or secondary to large tumours of the penis or may be encountered in the late stages of dourine. In dogs, paraphimosis may be observed as a result of stenosis of the preputial orifice usually following bleeding, excessive sexual desire or frequently without any apparent cause (Allam, 1953). Spinal diseases or injuries may cause paralysis of the penis in the male.

Treatment in bulls consist of cleaning the penis by removing the necrotic tissue and application of antibiotic ointment. Immediately after dressing, the penis should be pushed inside the sheath and vaseline should liberally be applied to prevent adhesions. Penis may be protected by providing a tight suspensory bandage.

*Chronic prolapse of prepuce:* This condition is most commonly observed in Bos indicus bulls and particularly in Sahiwal, Tharparkar, Deoni, Gir and Sindhi bulls (Sane *et al*, 1958). It is probably an inherited condition associated with a pendulous sheath and a large preputial orifice. The sheath is usually tucked up in draught breeds like Hallikar, Khillar and Malvi. The bull is unable to retract the prepuce and the erection of penis is not possible due to swelling. Urination is usually painful.

### Treatment

The prolapsed organ should be carefully washed, cleaned and dried. After applying antibiotic or bland antiseptic preparations, the prolapsed mass is replaced and held in place by pursestring

sutures. Even if treatment is done, there is every chance of recurrence.

### (c) MISCELLANEOUS CAUSES FOR INABILITY TO COPULATE

Conditions like scrotal and umbilical hernias may interfere with serving ability. Males with umbilical hernia should not be used because of its hereditary nature.

Loss of sensory innervation of glans penis prevents natural intromission and leads to pronounced decline in sex drive. Urinary calculi, lodged in the urethra may cause acute pain and thereby retard the sex drive (Vander Sluis, 1953). Prognosis is grave in this condition. Acute prostatitis in dogs is associated with pain and may cause failure to copulate.

The animals may refuse to serve in conditions like peritonitis, traumatic (pericarditis) reticulitis, seminal vesiculitis and acute orchitis.

## III. INABILITY OR REDUCED ABILITY TO FERTILIZE (IMPOTENTIA GENERANDI)

Impotentia generandi in the male is characterised by normal sexual desire and normal ability to copulate but the fertility is either low or absent.

The failure of fertilization or low fertility may be caused by defects in the semen, which are not recognisable in apparently normal semen.

### (a) Impotentia generandi with apparently normal semen

The semen ejaculated by bulls infected with vibriosis and trichomoniasis and some viral agents may be normal with respect to the routine semen evaluation tests like motility, percentage of live sperm and liveability; but such

samples may produce embryonic or fetal death leading to infertility.

Low fertility is also encountered in bulls with acrosomal defects like "knobbed sperm" though the motility is normal in these samples.

Infertility in certain bulls with apparently excellent semen was traced to intrachromosomal aberrations like translocations and inversions (Gustavsson, 1969).

Impaired fertility was also noticed in a group of stallions with apparently normal semen due to inherent enzymic differences (MacLeod, 1951).

Low fertility in some bulls with normal semen was attributed to low DNA (Leuchtenberger *et al*, 1956) but this is disproved by Gledhill (1966) who reported that the mean amount of DNA between fertile and infertile bulls was about the same.

There is no difference in seminal attributes in high fertility bulls and those with impotentia generandi condition. Such type of infertility in bulls has been detected by Knudsen (1958) in SKB breed of Sweden.

### (b) Impotentia generandi associated with abnormal semen due to pathology of genital organs

The pioneer work of Lagerlof has clearly established the relationship between semen characteristics and testicular pathology. The condition of the testis is reflected in the type of semen produced. A close relationship between semen characteristics and fertility also exists.

(i) The pathological conditions of the testis are classified as:

- (1) Cryptorchidism
- (2) Testicular hypoplasia

- (3) Testicular degeneration
- (4) Testicular inflammation
- (5) Testicular fibrosis and
- (6) Testicular tumours

## 1 CRYPTORCHIDISM

In most of the domestic animals testes are situated in the scrotum at or soon after birth. This takes place by descent of testes through inguinal canal. In some instances the testes may remain inside the abdominal cavity. When one or both the testes fail to reach the scrotum at the appropriate time the animal is termed cryptorchid and the condition is known as cryptorchidism.

The retained or cryptorchid testes are usually small in size, soft and flaccid

and do not produce any spermatozoa. Spermatogenesis is inhibited by the elevation of temperature of the affected testis. Cryptorchids should never be used for breeding as it is a hereditary condition.

## 2 TESTICULAR HYPOPLASIA

Testicular hypoplasia is congenital and hereditary in origin caused by single recessive autosomal gene with incomplete penetrance. This is characterised by lack or marked reduction of spermatogonia in one or both gonads during fetal life. This condition is observed in bulls, buffaloes, rams and stallions. The incidence of testicular hypoplasia is summarised in Table 45.

Table 45  
INCIDENCE OF TESTICULAR HYPOPLASIA

Country	Breed	Incidence	Reference
1 Sweden	SRB	23%	Lagerlof (1934)
	SKB	28.1%	Eriksson (1950)
2 Denmark	RED Dane	17.6%	Blom & Christensen (1951)
3 UK	RED Dane	17.8%	Haq (1949)
4 USA	RED Dane	3 out of 6 Sterile bulls	Fincher <i>et al.</i> (1942)
5 USA	Milk and Beef	146 out of 10 910 bulls	Carroll <i>et al.</i> (1963)
6 USA	Dutch	11.7%	VanDer Sluis (1953)
7 USA	Dutch	17 out of 20 Sterile bulls	Derieux <i>et al.</i> (1955)
8 Switzerland	Dutch	22%	König (1959)
9 India	Gir	9 Cases	Kodigali (1964)
10 India	Cross Bred Bulls	1 Crossbred bull	Rao <i>et al.</i> (1966)
11 India	Holstein Friesian Bulls	Occurrence reported	Deshpande <i>et al.</i> (1976)
12 India	Berari Buffalo Bull	Occurrence reported	Patil and Karkum (1975)



Fig. 129a Unilateral testicular hypoplasia  
(Left) in a bull.



Fig. 129b. Unilateral testicular hypoplasia  
in a bull.

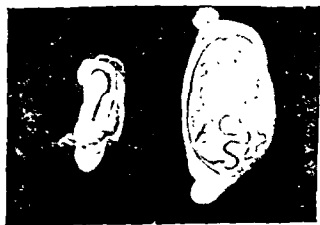


Fig. 129c. Unilateral testicular hypoplasia



Fig. 129d. Unilateral testicular hypoplasia  
in a bull.

Testicular hypoplasia may affect one testis (unilateral) or both testes (bilateral). This condition was thoroughly investigated in Swedish cattle by Eriksson (1913), Lagerlöf (1951) and Settegren (1961).

The degree of hypoplasia varies from partial to complete and only in bilateral complete hypoplasia the animals are sterile. The affected testis is reduced in size depending on the degree of involvement. The development of other genital organs is normal. In bilateral cases, both the testes are smaller than the normal size. Left sided hypoplasia is more common than the right sided. Sexual desire is not affected (Fig. 129a, b, c, d).

The semen picture is characterised by low concentration of sperm, low motility, higher incidence of proximal cytoplasmic droplets and abnormal spermatozoa. In bilateral cases, semen samples are clear and watery containing few or no sperm.

Histological examination of the testis reveals that the seminiferous tubules are lined by a layer of Sertoli cells and perhaps a basal layer of stem cells or spermatogonia, which do not show mitotic activity. The basement membrane is of normal thickness.

Prognosis is very poor if it is of hereditary origin and such bulls should not be used for breeding purposes. This condition is not amenable to treatment.

### 3. TESTICULAR DEGENERATION

This is the most common condition affecting the testis and is caused by a variety of factors.

Physical and traumatic causes such as excess of heat or cold, trauma, excessive physical strain, unilateral castration and irradiation may cause testicular degeneration.

accompanying fever and toxæmia may cause testicular degeneration. This condition is also caused in hot environment. Summer infertility in exotic bulls and rams is a common phenomenon. Degeneration is also encountered after insect bites on the scrotum and after dipping in arsenic solutions. Degenerative changes are also noticed with advancement of age. These changes are often accompanied by testicular calcification (Fig. 130c). Diseases such as Foot and Mouth and certain other viruses are known to produce degeneration in the testis. Testicular degeneration is commonly observed in exotic bulls after vaccination against diseases like Rinderpest and Foot and Mouth (Sharma, 1969).

Severe Vitamin A deficiency may also produce degeneration. In experimental conditions degeneration can be produced by insulation of scrotum or by application of hot water at 42°C to the scrotum for 1 hour or more. Degeneration has also been produced by injection of autologous or homologous testicular material with Freund's adjuvant (Roberts, 1971).

Signs of testicular degeneration: In affected bulls, there is lowered fertility. The size of the testis is not usually altered but in prolonged cases there may be slight reduction depending on the duration. The consistency is firm. Extensive adhesions between testis and tunica vaginalis are usually found. The libido of the bull is not altered.

The semen picture varies from very nearly normal to watery depending on the degree and duration of involvement of testicular tissue. The changes in the semen picture begin to appear from 10-50 days after the cause begins to act. The sperm cell concentration and moti-

lity are reduced. Increase in the incidence of loose heads is the first morphological sign of degeneration. The percentage of proximal protoplasmic droplets may vary from 30-60% depending on the degree of degeneration as against normal incidence of 2.5%. The incidence of abnormal sperm heads may vary from 30-50% as against normal incidence of 10-15% (Lagerlof, 1934; Gunn, 1936; Phillips and McKenzie, 1934; Rao, 1971; and Deshpande, 1972).

The tail abnormalities are not usually associated with testicular degeneration. Spermatocytes and giant cells are commonly seen in the semen.

Histologically, the tubules are reduced in size and the basement membrane is thickened. The thickness of the germinal cells decreases and the presence of pyknotic nuclei in the germinal layer is very common. There is considerable increase in interstitial tissue.

Prognosis is variable depending upon the causative factors as well as duration and degree of involvement of testicular tissue. Though degeneration can set in very rapidly, regeneration takes long time usually requiring 3-6 months or more. In mild cases the prognosis is favourable, in chronic cases the prognosis is poor. Severe cases of degeneration lead to fibrosis and the recovery is never possible.

Treatment consists of correction or alleviation of causative factors coupled with sexual rest. In nutritional deficiencies supplementation with Vitamin A is recommended. For exotic bulls and rams in tropical climate airconditioning or cooling is recommended.

#### 4. ORCHITIS

Orchitis denotes inflammation of testis. Besides abscess formation due to

trauma, orchitis is usually caused by *Brucella abortus* and some viral agents (Fig. 130a, b). A specific viral disease producing Epivag in African countries also produces orchitis; other agents include *Mycoplasma*, Psittacosis — lymphogranuloma venereum. Vaccination of young bulls with *B. abortus* strain 19 may produce orchitis.

In acute orchitis the scrotum becomes hot, painful and oedematous (Fig. 131). There is rise of body temperature accompanied by anorexia and dullness.

In acute orchitis sexual rest is useful. Antibiotic therapy is also indicated. Application of ice packs to testis may also help.

*Brucella* infected bulls should never be used for breeding as there is great risk of transmission of infection.

#### 5. TESTICULAR FIBROSIS

It is usually the end result of testicular degeneration. The testes are small in size and firm in consistency. Marked degenerative changes in the germinal epithelium together with increased interstitial tissue are noticed histologically. The semen is usually watery containing few or no sperm. This condition was observed in two Khillar bulls suffering from mucosal disease complex (Sane *et al*, 1963).

#### 6. TESTICULAR NEOPLASMS

These are common in dogs and old bulls but not in other animals. Interstitial cell tumours appearing as round masses are common in old bulls. The semen picture is not altered and the fertility may not be affected. Seminoma and Sertoli cell tumours have also been described in bulls.

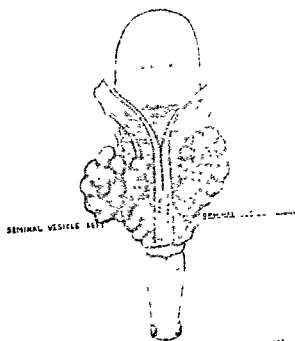


Fig 132 Spermiosclerosis in a ram



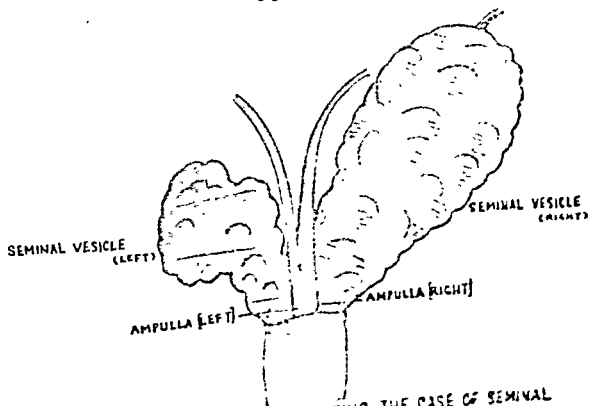
Fig 132 (a) Suppurative epididymitis in a buffalo bull

Note the enlargement of cauda



SCHEMATIC DIAGRAM ILLUSTRATING NORMAL SEMINAL VESICLES OF A BULL.

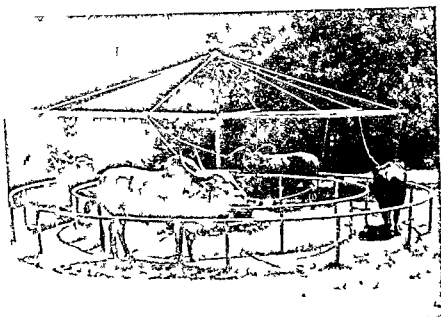
Fig. 133a:



SCHEMATIC DIAGRAM ILLUSTRATING THE CASE OF SEMINAL VESICULITIS (RIGHT) IN A BULL.

Fig. 133b:





Bull Exerciser

#### IV. THE AFFECTIONS OF EPIDIDY- MIS AND ACCESSORY REPRODUC- TIVE GLANDS

##### I. Anomalies of epididymis

*Segmental aplasia* of Mesonephric duct is a congenital hereditary condition most commonly seen in bulls. The body or tail or the entire epididymis may be missing. This condition is found to be common in Danish bulls (Blom and Christensen, 1951). In majority of the cases the condition is unilateral and the bull is fertile. This can be detected by palpation. In bilateral cases the semen is watery with few or no sperm.

There is no treatment and affected males should not be used for breeding as it is a hereditary condition.

##### SPERMIOSTASIS

This may be caused by blind, rudimentary mesonephric tubules, ductuli aberrant. This condition is common in bucks, rams and less common in bulls. This is probably of genetic origin. Patil and Kaikini (1975) recorded cases of spermiosstasis in Berari (Nagpuri) buffalo bulls (Fig. 132).

##### EPIDIDYMITIS — (Fig. 132a)

It is usually secondary to orchitis. The infectious causes are *Brucella abortus* in bulls, *B. suis* in boars and *B. ovis* in rams. Specific viral agent causing "epivag" characterised by bilateral hardening and swelling of the tail of the epididymis has been reported in African countries.

*Brucella ovis* is a common cause of epididymitis and infertility in rams in Australia. The organisms cause perivascular lesions with oedema and fibrosis in epididymis resulting in obstruction of the lumen and stasis of epididymal contents which leads to formation of

spermatoc granuloma (Deshpande, 1975).

Prognosis in severe or moderate epididymitis is poor.

##### 2. Vas deferens and ampulla

Segmental aplasia of seminal vesicles is usually accompanied by segmental aplasia of vas deferens and ampulla.

##### 3. Seminal vesicles

Segmental aplasia of seminal vesicles is reported in Danish bulls.

Seminal vesiculitis is the most common condition affecting the gland. The incidence varies from 3 to 4% in European breeds. Only few cases are recorded in India (Johari, 1957; Sane *et al*, 1965; Kaikini *et al*, 1968 and Kodagali, 1970). There are two forms of seminal vesiculitis. The first form is usually caused by *Brucella abortus* and *C. pyogenes*. Often it is unilateral. The affected vesicle is enlarged. Fibrous adhesions around the organ involving other structures are often detected. The semen contains flocules of pus, epithelial cells and leucocytes. The motility of the sperm is poor. The second type of seminal vesiculitis is still of unknown cause but it is the most common type. The causative agents may include chlamydia, mycoplasma and other viruses. This is often bilateral and the affected vesicle is not grossly distorted. An increase in consistency and change in the clarity of lobulation may be the only detectable change on palpation (Fig. 133a, b). This is rarely associated with peritonitis or adhesions. Semen contains purulent material, leucocytes, epithelial cells and chromatin plates. Motility is decreased.

Prognosis is fair to poor. Males suffering from Brucellosis, Tuberculosis or

Mycoplasmosis of seminal vesicles or those with secondary lesions of testis, epididymis and ampulla should be slaughtered.

*Treatment.* Antibiotic therapy may help. But in most cases seminal vesicles become fibrotic and indurated.

For selected bulls surgical removal of seminal vesicles is advocated (Galloway, 1964; Blom and Christensen, 1965).

#### 4. Prostate

Diseases of prostate are uncommon in all animals except Dog. Prostatic hyperplasia is most common in dogs over 5 years of age and castration is the most satisfactory method to reduce the size of the prostate (Karmalkar, 1973).

#### PATHOLOGICAL CONDITIONS IN THE BUCK

In bucks infertility or sterility is mostly caused by testicular or epididymal affections. An extensive study on the pathological conditions were undertaken by Mathew (1977). One thousand pairs of testicles and epididymis from bucks aged 6 to 18 months were collected from abattoir for studies. Out of the thousand pairs of organs examined 174 (17.4%) revealed various types of pathological lesions as under:—

(i) Testicular hypoplasia	5.8%
(ii) Cryptorchidism	2.9%
(iii) Ectopic testes	0.1%
(iv) Testicular degeneration	0.4%
(v) Adhesions with tunics	1.8%
(vi) Testicular atrophy	1.3%
(vii) Testicular hermaphroditism	0.1%
(viii) Male pseudo-hermaphroditism	0.1%
(ix) Spermiostasis	1.1%

(x) Spermatic granuloma	0.1%
(xi) Epididymal haemorrhage	0.4%
(xii) Epididymal Melanosis	4.3%

In addition to the above 258 organs i.e. 25.8% showed minor developmental aberrations such as Cystic persistent Mesonephric tubules (8.9%), appendix epididymitis 14.7% and Cystic remnants of Mullerian ducts, 2.2%.

#### EVALUATION OF BREEDING SOUNDNESS IN MALES

A careful and critical examination of a male is essential to assess his breeding potential. The principal steps in evaluation consist of:

1. Breeding history
2. General physical examination of the male
3. Special clinical examination of the reproductive system
4. Semen examination

##### 1. Breeding history

Breeding records are essential in the diagnosis of fertility. The conception rate, confirmation and presence of inherited defects of the daughters and records of the protective vaccinations undertaken should be considered.

2. The general physical examination should include information about the condition of its hooves, limbs and joints. The general condition, temperament and attitude of the animal should also be noted. A careful examination of the circulatory, respiratory, digestive and lymphatic system should be carried out for evidence of any disease.

### 3. Special clinical examination of the reproductive system

#### EXTERNAL EXAMINATION OF GENITALIA

The scrotum should be inspected to detect asymmetry and skin lesions. The scrotum and the scrotal contents are palpated with respect to the mobility of testes.

Epididymis is to be palpated for the existence of epididymitis and spermio-sistis.

The ampullae are palpated for their thickness.

The penis and prepuce are best inspected at service or at the time of collection with artificial vagina. If the penis is not observed at the time of service, it may be manually protruded for examination.

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tilation. The roof should be sturdy and which can easily be cleaned. Ventilation outlets are necessary. The corners of the walls be well rounded for better cleanliness and disinfection. The walls and roof should receive occasional white wash of lime alongwith the feeding mangers and watering troughs to maintain hygienic conditions. The floor should be of either rough concrete or vertically placed hard bricks with proper slope. Floors prone to become slippery e.g. stones, shahabad slabs and smooth concrete should be avoided. The runs should be provided with hard soil of at least 1 to 2 feet deep. Providing easy access to the run from the pen will ensure good exercise and will prevent overgrown and deformed hooves.

Breeding bulls should not be tied on standing for long time, else they may develop hygroma. The feeding troughs should preferably have an overhead semicircular shape to direct the feed stuffs to fall back in to the trough. This will avoid spoilage of feed. Water troughs should be always full with clean water.

Majority of the bulls are aggressive and furious. Daily handling by way of grooming, leading and exercising will however make them manageable. The breeding bulls of draught and dual type e.g. Khillar, Amritmahal, Kankrej, Malvi, Hallikar, Dangi, Ongole and Kangayam are usually aggressive. The Gir, Red Sindhi, Sahiwal, Tharparkar, Deoni and Haryana bulls are less aggressive. Bulls are easily manageable by persons familiar to them.

Use of copper or brass nose ring gives additional security in controlling them. A long strong hook of about 5' to 6' ready at hand is of great help to separate bulls during fights. Nylon nose

strings which are strong and durable are popularly used.

Bulls require special protection against crows which usually like to prick on their humps, tail heads or near the base of the horn to grab debris. Daily grooming of such bulls will prevent crow bites.

Coir gloves or body brushes are generally used for grooming the bulls. Grooming helps in cleanliness, better cutaneous circulation, freshening and developing association with the bulls. Inspection between the forelegs, inguinal region and the under surface of thigh, testicles and base of tail is necessary to keep them free from ectoparasites. Special care is necessary for the feet to check interdigital spaces for wounds and foreign bodies. Paring of overgrown hooves should be done occasionally. The overgrown tufts of hair at preputial orifice should be clipped.

Bulls, bull boxes and paddocks should be kept tick free to prevent Piroplasmosis and Theileriasis.

Exotic bulls can be easily led by putting a string to the nosering or by using a halter. Many of the indigenous bulls are found to be sensitive to handling by rings and it is therefore necessary to control them by nose-strings. Bulls should not be kept very close as fights may ensue.

### Exercise

Moderate exercise keeps the breeding bulls trim. Open runs or tethering grounds are the best means to exercise them. Bulls usually like to wander about, jumping and goading the grounds with their feet and horns. In the absence of open air paddocks a bull exerciser serves the purpose [Fig. A133]. Beneficial effects of exercise have been

reported by Bonadonna (1956). Exercise of 850 meter on days of collection and over 2000 meter on rest days showed substantial gain in ejaculate volume from 83% to 92% between age group of 3 to 9 years (Poroshin and Oboskalov, 1974).

### Health Protection

The breeding bulls should be tested against Brucellosis, Vibriosis, Trichomoniasis, Tuberculosis, Leptospirosis and Jone's diseases periodically. In addition to this, bulls should be protected by prophylactic vaccination against common contagious diseases such as Rinderpest, Foot & Mouth disease, Haemorrhagic Septicaemia, Black quarter, Anthrax and Theileriasis periodically. According to the seasonal occurrence of the diseases, a schedule of preventive vaccination programme should be arranged.

Bulls should be cleaned before semen collection. Antiseptic solutions should not be used for wash. Under no circumstances the prepuce and the under-surface be wet at the time of collection of semen. The site of collection should not be crowded particularly with unfamiliar people. Restraint, false mounts, back and forth movement of the teaser animal induce good sexual excitement in many bulls. Sexual preparation prior to collection improves the qua-

### Vices

Vices may develop on account of improper handling, close housing, confinement in dark, lack of exercise, abnormal surroundings and associations with castrated males.

#### (1) MASTURBATION

Masturbation has no significant effect on fertility but it may prove harmful. Irregularity of collection schedule is also a contributory factor. Such bulls can not be used with certainty.

#### (2) VICIOUSNESS

It is the outcome of confinement and also of ill treatment. Proper handling and exercise of bulls from young age will help to overcome viciousness.

#### (3) SLUGGISH BREEDERS

This can be attributed to low endocrine constitution. However, sluggishness may occur on account of improper training, rough or ill treatment or painful experience during service.

#### (4) LACK OF SEXUAL DESIRE

Primarily this is due to low endocrine constitution. However the following factors are also responsible in causing this condition viz. poor or faulty feeding, emaciation, deficiencies in total digestible nutrients, avitaminosis A, deficiencies of protein, phosphorus and cobalt, semistarved conditions during puberty, obesity and feeding excessive roughages.

purposes. However they cannot be allowed free wallowing on account of strong antagonism with each other.

Buffalo bulls require moderately cool housing in order to maintain optimum semen quality.

Inability to tolerate heat can very well be attributed to the lack of sebaceous and sweat glands and scanty hair (Mason, 1974).

The epidermis and corneal layers are thick in buffalo skin and may possibly act as thermal insulator against hot air or as a barrier against ectoparasites and possibly leeches.

Buffalo bulls may be shaved occasionally to keep them clean.

Housing of buffalo bulls should be on the same pattern as that for the cow bulls. They take easily to the bull exerciser but leaving them in open air paddock with plenty of shade is preferable.

Dietary requirements of buffalo bulls are similar to those in cow bulls.

Buffalo bulls are comparatively very ferocious as compared to cow bulls. A stainless steel nose ring should therefore be fitted. Dehorning will reduce the risk of goaring.

## CARE AND MANAGEMENT OF RAMS

### Housing, feeding and management

If rams are to be purchased for breeding, they should be procured well in advance before commencement of the breeding season for acclimatisation. It is a good practice to keep rams separate from ewes except during breeding season.

For housing rams a good airy shelter with well rammed dry ground annexed

with a well protected run will serve the purpose. It is advisable to provide fencing of thick sturdy galvanised wire upto 5' and two lines of barbed wire for about 2 feet above that to provide protection from dogs, cats and wild animals. Open runs provide ample exercise. However, rams may be left to pastures or fields during non-breeding seasons separately. Rams should not be allowed to become obese which reflects on their breeding efficiency.

Rams subsist largely on pasture and dry roughages. If grazing is scanty it should be supplemented with concentrates ( $\frac{1}{2}$  kg. per day). Lush green supplement helps to maintain general health and vigour. Vitamin A should be supplemented in case greens fall short. Concentrates should be specially fed 6 to 8 weeks prior to breeding season (flushing).

### Preparations for breeding

1. Conditioning by providing concentrates 6 to 8 weeks prior to breeding season.
2. Hooves should be trimmed to prevent injury during coitus.
3. Shearing will make the ram more active. Clipping from neck down to the prepuce should be done (crutching), for effective services.
4. Smear the breast and area between forelegs every day or on alternate days with thick oily paste non-injurious to wool. The ordinary washable dyes available in market may be used with common oil. Picric acid is also used. Oil paints or tar should be avoided. This device is useful in detecting ewes in estrus followed by the ram.

Ensminger (1962) recommended that the colour of dye to be applied on bris-



ket should be changed every sixteen days to detect repeat breeders. The order recommended is Yellow Ochre — Venetian Red and Lamp Black.

One ram should be allotted for 50 breedable ewes to have effective tupping.

A vigorous ram lamb may be provided with 20 to 25 services per year when hand mating is done. Yearlings or older rams may be provided with 50 to 75 services per year for hand mating and 35 to 60 services per year for pasture mating. A good ram should remain as a vigorous breeder upto 6 to 8 years (Ensminger, 1962).

Wounds should be attended to promptly to avoid complications. Shearing will help detecting injuries in good time. Deworming should be done periodically. The flock should be tested against brucellosis and vibriosis. Preventive vaccinations should be done against foot and mouth disease, enterotoxaemia, pox, H.S., Rinderpest and Blue tongue.

chaffed fodder. During breeding season the feeds should contain more of proteins, minerals, vitamins, bone meal and salt licks. Clean drinking water should be provided in the box and also in paddocks. During rainy season when stallions get less exercise, providing feeds such as carrots, wheat bran, linseed meal and plenty of fresh clean water will prove beneficial.

Exercise is most essential to keep stallions thrifty and virile. Light breeds may be exercised under the saddle or hitched to a cart, standard bred stallions dragged 3 to 5 miles while drawing a cart and thorough bred are best exercised for 30 minutes to 1 hour under saddle daily, especially during breeding season. Exercise should not be hurried but should be simple walks and trots. Stallions require very good attention to their feet.

Regular grooming keeps the stallion clean and healthy.

the sight of the stallion but they should stand in such a position from where they can watch the service.

### (iii) PASTURE BREEDING

This is seldom practised in domestic horses. The stallion is allowed to run with a band of mares. Defects noticed in this system are:

- (a) Likely to cause injury to male or female.
- (b) Repeated services to one particular mare deprives services to other mares in heat.
- (c) Stallion may become infertile by the end of breeding season on account of frequent services.
- (d) Recording of services becomes difficult.

### (iv) ARTIFICIAL INSEMINATION

A.I. method of breeding has a limited use at stud farms. No significant difference was noticed for sperm number, gel free volume, gel volume, pH and sperm motility when collections were made with or without false mounts (Cornwell *et al*, 1974).

One service a day is a satisfactory frequency in breeding. Occasionally a virile stallion may serve two mares in a day. A stallion remains virile upto 20 to 25 years of age. Pickett and Voss (1975) observed that mismanagement and rough treatment at service resulted in abnormal mating behaviour. Very frequent ejaculations during winter had a detrimental effect on the service behaviour of young stallions. Pain due to injury sustained during service may lead to reluctance to serve.

Masturbation is likely to occur on account of accumulation of smegma with subsequent irritation. Cleaning of sheath, good exercise and regular use at

service will help to prevent masturbation. Ensminger (1962) advises mating guide for horses as in Table 46a.

Table 46a  
MATING GUIDE FOR HORSES

No. of mating per year		
Age	Hand mating	Pasture mating
2 year old	10 15	Preferably no pasture mating
3 year old	30-40	
4 year old	40-60	
Mature horse	80-100	
Over 18 year old	20-40	

### Comments

Limit the 2 year old to 2-3 (matings) services per week, the 3 year old to 1 service per day and the 4 year old or over to 2 services per day. A stallion should remain a vigorous and reliable breeder upto 20 to 25 years of age.

### Accidents during service

Due to kicking by mares in oestrus the following accidents may be caused to the stallions; injury to penis, ventral hernia, fractures of hind limbs, orchitis and inguinal hernia.

### Vices

- (i) Viciousness due to improper handling.
- (ii) Stall walking, weaving, cribbiting, self biting or mutilation.

### Remedial measures

#### (i) STALL WALKING

This can be prevented by regular exercise, hobbling or putting bales

around in the stall or by putting the stallion in a paddock.

(ii) CRIBBITING

This can be prevented by cribbing strap or by surgery.

(iii) WEAVING

This is difficult to correct but can be overcome by proper exercise or by providing a large well lighted loose box or a roomy paddock.

(iv) SELF BITING

Prevented by cradle.

### MANAGEMENT OF BOAR

Roomy, well ventilated, hygienic pens should be provided for housing boars. For proper exercise well fenced runs of about  $\frac{1}{4}$  acre per boar are necessary. Water should be provided adlib. Boar should normally receive  $\frac{1}{2}$  to 1 kg. of ration per 50 kg. body weight. Body weights should be checked regularly to detect optimum condition, fatty tendency or leanness. Boars do require good quality of hay and greens.

Hand mating is generally practised (Table 16b). Use of crates for heavy boars may be tried but many a times

this may disturb the boar. Dubiel (1974) recommends that boar should be kept in individual pens for at least 48 hours before semen collection.

A boar can become ready for service at the age of eight months. First start with one service a day with a total of 24 services in a breeding season.

A strong vigorous boar 1 to 1 years of age may serve 2 sows per day during breeding season provided that a system of hand coupling is practised.

### Vices

(1) Some boars pace back and forth along the fence, chopping their jaws and slobbering; this action is termed as "Ranting". Young boars habituated to this may result in poor development.

(2) Viciousness can be minimised by clipping of tusks well in advance of breeding season.

(3) Some boars develop habit of masturbation in preputial diverticulum which is called as "balling up". An operation to prevent this is recommended by Roberts (1971).

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PART IV

**SEMINOLOGY  
AND  
ARTIFICIAL INSEMINATION**

Veterinarians in the technique. Millions of cattle and sheep were inseminated in Russia as early as 1930, which gave sufficient proof of its success and it opened a new avenue for research in livestock development.

In Denmark, Sorensen (1938) of the Royal Veterinary College did the earlier spade work and introduced this method in cattle breeding through the network of co operative organizations.

In America Dr J. A. Henderson introduced this method by starting work at Minnesota in 1931. This method of breeding is now being widely used through a net work of co-operative organizations.

In Great Britain, Dr. J. Hammond and Dr. Walton undertook extensive studies on Physiology of Reproduction in cattle. The first Artificial Insemination breeding centre of the Ministry was established in 1942 at Sonning near Reading under Dr. Stewart as the Director. Real impetus to the work was given by Dr. J. Edwards by establishing a number of A.I. centres under the Milk Marketing Board of England.

Credit goes to Professor Nils Lagerlof and Prof. Eriksson of the Royal Veterinary College, Stockholm, Sweden in enlightening the Veterinarians and the Stock breeders on the importance of the sexual health control to check the incidence of infertility in the herds. They also cautioned on the hazards that are likely to occur by use of substandard bulls. They have specially stressed the importance of hereditary influences. Attention was first focussed by them on the importance of progeny testing and rigid selection of bulls.

In Sweden, with the establishment of Artificial Insemination Organization,

the reproductive capacity of bulls has become the subject of increasing interest. The Animal Breeding Institute at Wiad during the year 1936-1943, conducted several experiments to study various problems. Early progeny testing of bulls was also undertaken at the initiative of Prof. G. Bonnier for selection of bulls of proper traits. Dr. A. Bane and Dr. H. A. Hultanas did extensive investigations on sexual behaviour of bulls and brought to the notice of the world that defective bulls may cause considerable damage due to undesirable hereditary traits.

In Norway, Artificial Insemination in cattle was first started in 1952 and work was managed through A.I. and Breeding Associations.

In India, Artificial Insemination was first attempted in 1939 by Dr. Sampath Kumaran. Credit goes to Dr. P. Bhattacharya of I.V.R.I., Izatnagar and his team consisting of Dr. S. S. Prabhu, Dr. S. N. Luktuke, Dr. A. Roy, Dr. D. P. Mukherjee and Dr. Gajjan Singh for undertaking comprehensive studies on problems of Artificial Insemination such as cytomorphology and physiology of spermatozoa, biochemical studies, semen extenders, effect of nutrition and exercise on sperm production, seasonal effects on reproduction and reproductive physiology of Indian farm animals. They also trained Veterinarians from most of the states of India in the technique of Artificial Insemination.

In most of the states in India, Artificial Insemination work was started between 1918-1954. The real impetus was received from 1951-56 due to launching of master project of the Key Village Scheme which envisaged allround improvement of cattle and buffaloes in the country. Artificial Insemination was accepted as a major activity of this scheme

for bringing about rapid genetic improvement.

At the recommendations of the FAO Expert Nils Lagerlof in 1953-54, Cattle Sterility Schemes were started in most of the states in India. The FAO conducted several advanced courses in Animal Reproduction under his able guidance. Sexual health control in various herds has thrown a good light on the breeding problems. Occurrence of vibriosis, trichomoniasis and trace element deficiencies was placed on record for the first time under the schemes in Bombay, Calcutta and Patna. Prof. Lagerlof has shown that there is a dire need for combining sexual health control service with Artificial Insemination.

In order to improve the productive capacity for milk, cross breeding schemes have been implemented in most of the states in India by obtaining frozen semen of progeny tested exotic bulls chiefly from U.K., U.S.A., Canada, Denmark and Australia. The main emphasis is being placed on Friesian and Jersey.

### ADVANTAGES OF ARTIFICIAL INSEMINATION

It has amply been proved through livestock development plans particularly for cattle and buffaloes that the method of breeding by Artificial Insemination has distinct advantages over natural breeding.

Importance is realised since it has been possible to make extensive use of superior sires for a very large number of conceptions. By adoption of this method it has become easy to have the sires progeny tested in good time. The use of outstanding progeny tested sire can thus be extended to a large number of females.

It has been possible through Breeding Associations in several countries to exercise rigid control particularly on the sexual health of sires and more particularly on breeding bulls to confirm that they are free from coital infections, e.g. Vibriosis, Trichomoniasis, Leptospirosis, EpiVag, Brucellosis and Tuberculosis.

Young bull calves brought over to Breeding Associations can be placed under optimum levels of nutrition and efficient managerial schedule so that, their growth rate is progressive until they reach puberty or sexual maturity.

This method particularly holds good in upgrading the stock with farmers owning a few animals and who can ill-afford to maintain a progeny tested sire just for the services of their few cows. In sizable herds though it is advisable to keep one or two bulls for natural services the farmer can ill-afford to maintain highly pedigreed and progeny tested bulls on account of the prohibitive cost. Moreover in breeding season it is not advisable for the farmer just to depend on one or two bulls for the probable services of a number of cows coming in heat on one and the same day or on successive days. Due to the widespread use of this method coital infections can be brought under control. This has resulted in considerable lowering of the high annual incidence of abortions with more number of normal calvings which ultimately counts towards the economics of the dairy industry. This method has therefore become inevitable in all the developmental plans for cattle and buffaloes.

In breeding young heifers difficulty is usually experienced in natural service if the bulls are heavy. Artificial Insemination has helped to overcome such diffi-

culty experienced due to size difference In superior cows with minor accidents to pelvis or hind legs breeding becomes possible through Artificial Insemination as against natural mating when it is impossible to use bulls for services It is possible to control seasonal reproduction by spacing the services through Artificial Insemination This is not possible if bull is running with the herd for natural service

Due to Artificial Insemination method of breeding it has become possible to examine every cow at close quarters to understand her reproductive health intensity and the type of oestrus if oestrus discharge is clean and if ovaries are functioning properly with progressive development of follicles and if the heat is ovulatory or anovulatory This is not possible in natural service It is possible to maintain correct records of breeding and check up the cows in good time if they do not conceive or if they become infertile due to one reason or the other

In the cross breeding of cattle Artificial Insemination method has an important place

Evaluation of semen is possible when semen is Artificially collected and rigid control can be exercised on semen quality Only the best semen samples with heavy concentration of viable spermatozoa are used and as such one is very sure during insemination as to the quality and viability of the semen used Conception rates are likely to be higher as compared to natural matings in which the bulls are always under stress during breeding season when they are supposed to cover a number of females in a limited span of time resulting in low conception rates In Artificial Insemination method of breeding the farmer is always assured of quality semen in and

out of the season and for any number of cows that may come on heat on a particular day

It has become possible to extend the quantity of neat semen by diluting it with suitable dilutors to obtain very large number of conceptions This is not possible in natural service

It has also become possible to preserve the semen under refrigeration conditions for a longer duration This has facilitated its easy transport to far off places This has given impetus to upgrade the stock in nook and corner of the country

Inseminations can be done right in the byres which has saved lot of trouble to the farmer in either taking the cow to the bull at far off distance or getting the bull to his premises Any possibility of catching infection during transit can thus be avoided In general it is highly profitable to the farmer in availing the Artificial Insemination service for his cows than to spend over a comparatively inferior type of bull It can thus be gathered that Artificial Insemination is a golden tool for rapid improvement of stock for economic traits

### LIMITATIONS OF ARTIFICIAL INSEMINATION

A very great care is necessary in the method of breeding by Artificial Insemination since careless approach may lead to disastrous results Hygienic precautions are necessary

Bulls free from coital infections have to be selected

Infected bull always proves dangerous since it will transmit infection to a number of cows which are inseminated from its semen as against only one or few from natural service



A battery of veterinarians and technicians trained in Physio-pathology of Reproduction and in Artificial Insemination technique is necessary to man the work. Care has to be taken at every stage such as handling bulls, collection of semen, its processing and storage in the laboratory, dispatch to the field, insemination work and the subsequent check up for pregnancy diagnosis or subfertility and infertility. Mistakes in channelising the work may prove costly. There is inherent danger of contaminating the semen lots at a number of stages right from collection to its deposition in the genital tract. Accidents are likely to occur when animals are being secured for insemination purposes. Similarly injuries by insemination pipettes to vagina and cervix and possible danger of carrying infection from the vulval opening by the pipette to the cervix is likely to occur. The inseminators need to have good knowledge of early pregnancy diagnosis in order to avoid accidents especially in cases of gestational oestrus.

Genetic abnormalities are likely to be propagated through bulls and as such great care is necessary to watch the progeny otherwise a large number may be born with variable defects. Expenditure has to be incurred on laboratory and Artificial Insemination equipment and on freezing facilities. A heavy cost is also involved on transport since technicians are expected to inseminate animals right in cattle sheds.

In case of an outbreak of cattle contagious disease particularly the foot and mouth disease, the work of the Breeding Association is paralysed. There are also possibilities of the infection being carried from farm to farm on account of the movement of the technicians.

In certain under-developed countries illiteracy among farmers, religious taboos and sentiments also serve as limiting factors. Communicating systems through proximity of good roads limits the scope of expansion of the activities. Similarly extreme climatic condition is also a limiting factor.

It can thus be gathered that the method of Artificial breeding is a very expensive one, but considering the numerous advantages, in the long run it always outways the limitations.

Developments achieved by the Milk Marketing Board, England and similar institutions in other countries in producing proven sires and the extensive use in augmenting milk production in the progeny is worth consideration.

The completion of 50 million first inseminations by the Milk Marketing Boards A.I. service in England is a remarkable achievement and an unique event in the world of cattle breeding (MMB Report 1981). The MMB organisation to-day operates the world's largest A.I. technicians service. "It completes almost 2 million first inseminations annually, has a stud of 1,000 bulls and offers service from over 30 different breeds. In 1981, it reached the landmark of 50 million first inseminations, before any other A.I. organisation".

At the heart of the MMB involvement also lay the hope that the use of A.I. with superior sires would increase milk production. The farmers were made to realise that what was urgently needed was an increase in individual cow production through improved breeding. It was also realised that this sort of scientific service by the MMB is a National need if any appreciable rise in milk production was to be achieved. Initially the MMB was involved in the

milk recording project which is the very basis of successful breeding programme for selecting high grade sires on production basis and type

### Breed Changes

In UK AI not only gave access to a superior sire but also to one of a different breed and the incidence of cross breeding was high. The accent shifted to Holstein Friesian and Jersey the choiced breeds of the world for milk production.

MMB has also played a great part in the introduction of many beef breeds such as Hereford Devon Aberdeen Angus Lincoln Red Sussex Charolais Limousin Murray Grey and Simmental

### Bull Selection

Every year MMB completes over 80% of total inseminations in England and Wales. This is entirely dependent upon the well organised and reliable progeny tested programmes. MMB responds to this responsibility by carefully selecting and thoroughly testing each of its BULL POWER sires.

### The Contract Mating Scheme

The search for suitable bull mothers begins at the MMB herd office where genetic indices for fat and protein production are produced for every recorded cow in England and Wales. The selected cows are then analysed for confirmation.

Of the thousands of cows inspected only 350 are chosen each year to produce about 120-140 bull calves needed to meet the requirements. Confirmation standards are therefore very high. Once a contract has been signed the cow is inseminated with semen from a specially selected sire from the current

contract mating list and the MMB guarantees to purchase the calf if it is a bull.

### Bull Rearing Unit

The newly purchased calves are taken to the MMB young bull rearing unit at the age of 10 weeks and reared there until one year.

### Dairy Progeny Testing Scheme

At 1 year the young bulls enter the dairy progeny testing scheme to analyse their ability to transmit both production and type to their daughters.

Semen collected from each young bull is used to inseminate around 330 cows in officially milk recorded herds throughout England and Wales. The aim is to have at least 50 daughters of each bull completing their first lactations to allow a reliable Improved Contemporary Comparison (ICC) to be calculated.

### The ICC

It is a sound indication of a bull's genetic ability to transmit production of milk fat and protein to his daughters. Management season and age factors are eliminated as well as the varied genetic level of contemporaries. However, it is stated that a good ICC is not sufficient on its own to guarantee a place in a proven bull power stud. Daughter confirmation is also taken into consideration. Every DPIS heifer is inspected by livestock officers. They are also analysed for ease of milking and temperament. With a proven sire being used up to 100 000 inseminations every year, his detailed analysis is carried out. When the test information is complete only 1 in every 8 DPIS bull will return to the proven stud.

### Lay-off Units

Bulls awaiting their DPTS results are kept at the lay off units. No collections are made at these units.

### Deep Frozen Semen

The greatest advance in A.I. since its inception was the discovery of a method of deep freezing of semen. The impact of the discovery was immense. Young bulls could be tested more quickly and top sires could be utilised fully. Larger areas could be had for operation and daily cost on transport of fresh semen reduced considerably.

MMB is now exporting semen to over 30 countries in the world. Since 1970 MMB has completely switched over to the use of frozen semen.

### A.I. Centres

MMB has 23 A.I. Centres in England and Wales with a network of sub-centres. Each main A.I. centre has upto 74 inseminators providing the year round service. Each centre and sub-centre operates a 24 hour telephone service with overnight calls recorded on an answering machine for the staff to act on the following morning. About 20 inseminations daily are done by

### Controlled breeding

In 1975 research with drugs to synchronise oestrus in cattle was taken up by MMB. Many a dairy and beef herds are taking advantage of this service frequently in England and Wales.

### Embryo Transfer

It marks the most recent advance in cattle breeding and its commercial application is the culmination of many years of MMB involvement in research.

The embryo transfer service launched by the MMB has created an important complement to the A.I. service for, where A.I. exploits the breeding potential of superior sires, so embryo transfer greatly increases the number of calves which can be obtained from the top cows. This development benefits both the farmers and the MMB. The farmer obtains heifer calves from superior cows while the MMB improves its chances of obtaining bull calves from contract mating cows for the dairy progeny testing scheme. The perfection of the embryo transfer technique will greatly ease the problems of synchronisation, storage and shipment.

# Chapter 39

## Artificial Insemination in Cattle

In dairy cattle development artificial insemination is widely accepted as a method of breeding for bringing genetic improvement. This method is extensively used for crossbreeding of the indigenous cattle with exotic blood in most of the developing countries. For successful implementation of artificial insemination in cattle the knowledge of reproductive systems of the male and female, hygienic collection of semen, its evaluation and sterilization of equipment is necessary. In addition to this training of the technicians and adoption of sound breeding and managerial practices by the farmers are equally important.

### COLLECTION OF SEMEN

#### Preparation of the Bull

The bull centre should have a schedule to prescribe days for collections from each bull. The usual practice is to collect semen from a bull once a week. However, two or even three successive collections may be obtained from the same bull. From young bulls collections may be obtained twice in a week.

#### Time for collection

Semen collections should be made during early morning hours before

feeding is done. Collections should as far as possible be avoided after feeding or during afternoon hours. Number of bulls are reluctant to serve on full belly. During morning hours the bulls are alert and fresh. Another advantage is that if the semen is collected during early hours, fresh lots can be used the very day which may raise the fertility rate to a certain extent.

#### Selection of a Teaser Cow

It is necessary to have a teaser cow on which the bulls are expected to mount during collection of semen artificially. As far as possible the cow should be of the same breed, size and colour. This is in view of the fact that the bulls will not find difficulty in reaching the back of the cow after mounting due to the reasonable height of the breed. Teaser should be of medium height and neither be too tall or short. In such extreme cases the bulls are either reluctant to mount or experience difficulty in mounting. The teaser cow should be physically strong and docile so that she is able to bear the weight of the bull easily during service and does not show irritable temperament when she has to stand in the service crate for a long time for collection of semen from a number of bulls. It is better to select a

cow which has done 2 to 3 calvings. Such cows usually have quieter temperament. She should be free from genital infections so as to be safe and that even if by accident the bull does natural service he should not catch any infection. She should also be protected against cattle contagious diseases. A teaser cow in heat should not be used as a teaser until the oestrus is over owing to the fact that cow in oestrus may normally stimulate the bull but there is every chance of his refusing to serve on an oestrus teaser cow on subsequent occasions. A pregnant cow should not be used as teaser. It is also not advisable to use a recent calver as teaser until about a month or so by which time there is cessation of the lochial discharge. An ovariectomised cow is recommended.

pairing of the hooves occasionally may keep the feet well trimmed and levelled. This consequently helps to maintain the normal gait and the bull will be at ease during mounting. The interdigital space should always be checked for foreign bodies. The person leading the bull should be well acquainted with the bull so that he does not cause any fright when bull is led to the service crate.

#### **Preparation of an Artificial Receptacle for collection of Semen**

avoid excess which might get mixed with the ejaculation. The first 7 to 10 cm of the opening of the A V should be lightly lubricated for easy introduction of the penis. If too little water is used and if air pressure is also not adequate ejaculation will not be normal. If the amount of water or pressure is excessive it may act as hindrance for the thrust resulting in no ejaculation. Bulls vary in their likes with regard to pressure in the A V. Certain bulls only react to high temperature in A V. A temperature above  $46^{\circ}\text{C}$  may kill sperm in the ejaculate. Bulls may refuse to serve if the temperature of A V is high. Very low temperature may fail to induce ejaculation. The valve should be adjusted to prevent escape of water and then air should be blown in through the valve to induce proper pressure. The inflation of air should be done in such a way that the opening of the artificial vagina should close resulting in bulging out of the inner lining to form a cushion. Insulation bag may be fixed on the graduated glass tube to avoid temperature shock. This is particularly required in cold season (Fig 134).

### Semen Collection Room

A quiet place should be selected for semen collection. A very spacious dust free room (12 × 15 meters) is preferred for semen collection. The flooring should be in plane with the surface

ground and hard murum should be spread over and well compressed to avoid dust. Water should be sprinkled in the service shed before collection. Concreting floor in the service shed should be avoided. Rubber matting may be provided in the rear to prevent slipping. It is ideal to fix the service crate at one end of the shed. The crate will fix the teaser cow in position restricting side movements during collection.

When teaser cow is fixed properly and artificial vagina is ready the bull should be led to the yard and then to the service shed. Veterinarian and the attendant should not use gaudy colour clothing as it distracts attention of the bull. The Veterinarian who is to collect semen should stand close to the right side of the teaser cow facing the bull. When the bull is mounting it should not be disturbed by the operator with his movements. The bull should only be permitted to mount when there is proper erection of the penis. Premature mounting may lead to incomplete thrust and ejaculation.

When mounting occurs on full erection the penis is deflected by grasping the prepuce and directed into the artificial vagina. Artificial vagina should not be forcibly applied on erected penis. The glans should not be touched by hand which in most bulls would cause disruption in mounting. After the thrust reflex A V should be withdrawn.

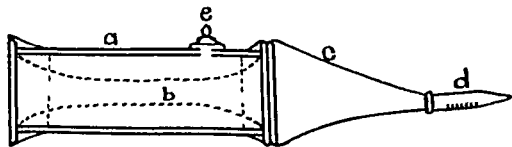


Fig 134 Artificial Vagina used for Bull.  
(a) Rubber cylinder (b) Liner (c) Cone (d) Collection tube (e) Ventil for water and air

## Other methods for collection of semen

### 1. ELECTRO-EJACULATION METHOD

The method was devised by Gunn (1936) for electrical stimulation of ejaculation in the ram. The method was further modified by Laplaud and Cassou (1918) for use in the bull. They used a bipolar rectal electrode. Thibault *et al* (1918) used a single electrode with the series of 30 rings thereon connected to the potentiometer. The apparatus gave a current of 50 cycles per second. Rowson and Murdoch (1951) further modified the apparatus by using two simple finger electrodes which were introduced in the rectum by a rubber

bulls of 16 to 19 months of age on alternate weeks for eight weeks by the electro-ejaculation and the artificial vagina methods. The resulting differences in percentage of motile, live or abnormal sperm were not found to be significant. However the average sperm concentration, total number of sperm per ejaculation, fructose and citric acid levels in the semen obtained by electro-ejaculation were significantly lower as compared to semen collected in the artificial vagina. This method is being rarely used on account of the discomfort caused and variable reactions.

### 2. MASSAGE METHOD

### 3 COLLECTION ON DUMMY COW OR MANIKIN

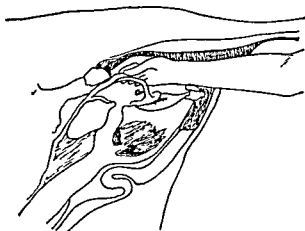


Fig 135 Semen collection by massage of ampullae of bull  
(a) ampullae

Injury to the rectum is likely to be caused if this method is used for semen collection too frequently

In about 5-10 minutes of ampullar massage usually results in the discharge of semen. Miller and Evans (1934) observed in 100 trials on 18 bulls that when seminal vesicles only were massaged they obtained seminal fluid containing epithelial cells and debris in every ejaculate. However, spermatozoa were present only in six of the trials. While as by massaging the ampullae they obtained spermatozoa in 91 out of the 100 trials. The quantity of ejaculate obtained on massage of the vesicular seminales and that from the ampullar massage ranged from 0.5 to 23.0 ml. The ampullar semen collected by massage is likely to be contaminated with urine. There is also high proportion of seminal vesicle secretion or it is likely to be imbalanced in its composition than the natural ejaculation. As such the seminal vesicles should not be massaged.

Semen collected by the massage method is likely to have low sperm concentration and high bacterial contamination than in samples collected with the A V (Hafez, 1968).

This is prepared of strong heavy steel frame and covered properly with hard numda over which a leather is fixed to give appearance of a normal cow. An artificial vagina is fixed at suitable height in the rear of the dummy. The A V is prepared in the usual manner and fixed in position when collection is to be made. Majority of the bulls do not show much interest in such dummy cows probably since the structure is lifeless. Moreover the A V cannot be fixed in proper direction of the penis when the bull is doing the thrust act. This acts as a sort of obstruction and the bulls lose their thrust reflex. Moreover there is likelihood of injury to the penis if it is not properly directed in the artificial vagina. On account of the several complications this method has not become popular.

#### Pre collection stimulation

Studies show that stimulation of a bull prior to collection results in increased ejaculate volume and percentage of motile spermatozoa by restraining the bull for few minutes before they are allowed to serve in the artificial vagina (Anderson 1945, Hellstrom 1947). In a more critical trial for over two months with identical twin bulls Crombach *et al* (1956) found a 100% increase in the number of motile spermatozoa when the bulls were restrained for about 5 minutes prior to collection followed by allowing them to have a false mount before obtaining the first collection. Kerruish (1955) reported on his trials that he obtained a conception rate of 60.8% with 10 bulls over a period of 5 months as compared to that of 68.5% when pre-collection stimulation was carried out.



### Frequency of Semen Collection

For all practical purposes the maximum collection frequency compatible with satisfactory conception rate is of great economic importance. Baker *et al* (1955-b) subjected nine bulls to study the effect of frequency of collection for one year after puberty with ejaculation frequencies of one, two or three times per week and noted that there were no effects on the volume of semen, spermatozoal concentration, percentage of viable or abnormal type of spermatozoa or on the total spermatozoa per ejaculate. Van Demarck (1956) showed that an increased frequency of collection combined with careful attention to pre-collection stimulation resulted in an increased number of spermatozoa. He further observed that partial semen exhaustion test at the intervals of 1, 4 and 7 days indicated that there was a rapid replenishment of spermatozoal reserves during the first 24 hours after collection and a steady rate thereafter upto 7 days. Bratton and Foote (1954) observed no effect on the fertilizing capacity or on conception rates with a schedule of collection one every four days or one every eight days over 272 days period. Two collections every eight days approximately gave 60% more motile spermatozoa than with the programme of one ejaculation every eight days with the same bulls. Almquist *et al* (1958) observed that after several successive ejaculations, there is a fall in the numbers of ejaculated spermatozoa but the fall is not below a certain low threshold level. Even after the collection of 11 to 30 successive ejaculates the spermatozoal replacement was found to be completed within one week. Frederick (1958) reported his findings on identical twin bulls that 4 collections per week compared with 2 per week reduced semen volume and

sexual interest although there was an 88% increase in spermatozoa production per week with no adverse effects on fertility. Hafez *et al* (1959) reported on his trials with one collection every day over a period of 8 months that there was no adverse effect on semen quality or fertility.

Lagerlof (1955) in his studies on Indian Zebu bulls recommended that collection should be made once a week. Singh (1960) observed no significant difference in semen characteristics of Kumauni hill bulls between one and two ejaculates every week. Sane *et al* (1954) observed that more than two collections per week in Sindhi and Gir bulls resulted in small volume, immature sperm in the ejaculate and decrease of sex desire. In the Indian draught breeds however, collection thrice or four times a week particularly during fair season did not affect the semen quality and fertility in bulls.

### FACTORS AFFECTING SEMEN PRODUCTION

In a breeding association it is essential to maintain bulls in prime condition. Bulls with high endocrine constitution will respond easily for collection of semen in the artificial vagina and the ejaculates in general show good promise for high fertility. The degree of sexual interest and ability to serve should not be considered as a correct index of fertility (Lagerlof, 1934; Anderson, 1939). However, certain number of slow serving bulls are known to produce semen of high quality resulting in high conception rates.

After sexual maturity maximum semen production and reproductive capacity are usually reached by 1 to 7 years of age and then it declines. Full sper-

matogenesis has been noted at 9 months of age. Testes show a relative decrease in weight after about 2 years of age and an actual gradual decrease after 5 years of age. Bulls upto 6 months of age secrete more androgen per gram of testes than older bulls (Nalbandov, 1961).

The continental breeds reach sexual maturity at an early age of about a year and a half while as, in the indigenous breeds of cattle in India the average sexual maturity in bulls is usually between 2 to 3 years of age. As age advances to that of 8 to 10 years the indigenous breeds show a lesser frequency and libido comparatively. Sperm concentration however, is usually normal until 8 years.

### Effect of Transport

Long distance transport of breeding bulls may lead to increased excretion of neutral steroids in the urine, subsequently followed by morphological abnormalities in the spermatozoa and decrease in the libido (Meschaks, 1953). Sane *et al* (1954, 1967) observed in Gir and Amritmahal, cow bulls and Murrah and Surti buffalo bulls that there was complete loss of libido in the Gir for months together and decreased libido in Murrah and Surti buffalo bulls after transportation over a long distance. However, the effect was transitory and not of permanent nature. Hafer and Bonadonna (1958) found that the average semen volume of 22 Friesian bulls, imported from four different countries varied with the climatic conditions.

### Seasonal effect

Seasonal effect on spermatogenesis conditioned due to hours of day light is claimed by Anderson (1945) and Mercer (1946). Temperature variation was found to be of less importance in their

studies. In Canada, Burgess (1953) reported no significant monthly or seasonal differences in conception rates. Schlindler (1951a) reported that in Israel conception rates were lowest in September during which period the lowest sperm concentration and survival rates were recorded. Schmidt (1951) reported that day light hours and temperature could be correlated with changes in the semen quality and also with fertility. However, when the seasonal differences in the atmospheric temperature and day light length are small, a marked change in the quality of semen and also on fertility can be expected. Johnston and Branton (1953) reported marked seasonal differences in the quality of semen over a period of one year. But there were no corresponding significant differences in non-return rates. By subjecting bulls to high environmental temperature (70-99°F), Casady *et al* (1953) obtained an adverse effect on the quality of semen. No change in semen production or fertility was recorded to varying conditions of atmospheric temperature and humidity (Patrick *et al*, 1959). Erb and Waldo (1952) reported that the conception rates on the non return basis were highest in September to November and lowest from January to April. Due to varied climatic conditions in India, it is observed that in heavy rainfall tracts in the coastal areas (1,500-3,000 mm), where humidity is high (80 to 100%) during monsoon season, breeding bulls show lack of sex desire and deterioration in semen quality. Under extreme climatic conditions of summer especially in northern India (105°-115°F) where exotic bulls are introduced for the first time, find it extremely difficult to adapt to high weather temperature in consequence of which the summer stress and deterioration of semen quality is invari-

ably noticed. In places where conducive arrangements are made for the bulls the semen quality is found to be normal.

### Genetic Effect

Influence of inherited factors with regard to poor serving ability and poor fertility has been reported by Lagerlof (1950). Lack of libido and sexual desire reported in Aberdeen Angus bulls by Couttie and Hunter (1956) was attributed to genetic effects but methods of rearing were also considered to be partly responsible.

### Effect of Exercise

Exercise just before collection results in the decline in the volume of ejaculate (Prabhu and Guha, 1953). They observed in their studies that bulls which did not receive exercise showed an increase in the pH and abnormal sperm, whereas, there was no difference in the quality of semen in bulls getting one or two hours of exercise. Sane *et al* (1954) observed that regular exercise to bulls either in the open air paddocks or by use of bull exerciser helped to maintain the bulls in trim condition.

produced in the testes and stored in the epididymis, (ii) seminal plasma, the secretion from accessory glands which is mixed with the spermatozoa at the time of ejaculation. Hormones from testes and pituitary control semen production. The components of semen and the metabolic changes have a great bearing on the dilution and preservation of semen.

### Composition of spermatozoa

#### HEAD

Mann (1951) has described the structure of spermatozoa in detail and stated that the head portion is mainly composed of Deoxyribonucleic acid (DNA). Mann (1951) reported that ribonucleic acid is not found in mature spermatozoa but it is present in spermatogonial and spermatocyte cells. Boguth (1952) observed the presence of plasmalogen (0.3 to 0.9 mg/ml. of semen) in the bull semen. They showed that one third of the plasmalogen is found in the seminal plasma which influences the survival of spermatozoa. Hartree and Mann (1953) observed that the lipid content in the ram spermatozoa is mostly plasmalogen.

Table 47

## BULL SEMEN NON-ENZYMIC COMPONENTS:

Results are average values [range in brackets] expressed in mg/100 ml. unless otherwise stated. An asterisk(\*) indicates that the analysis was carried out on seminal plasma and not on whole semen

Ammonia	2	
Adenosinetriphosphate NH <sub>2</sub> N	0.1	
Adrenaline	0.1	
Albumin	0.25*	
Ascorbic acid	6.0	(3.9)
	8.7*	
	7.6	(8.24)
Aspartic acid	0.09*	
Boron	1.71, 1.73*	
Calcium	34	(21-15)
	26, 17, 28*	
	37*	(21.60)
Carbon dioxide (bicarbonate)	16	
(ml/100 ml)		
Chloride (Cl)	371	(309-133)
	287.1, 320*	
	171*	(110-293)
Citric acid	720	(310-1150)
	620*	(357-818)
	726	(521-902)
	806*	(567-1000)
Creatine	3	
Creatinine	12	
Lipothionine	trace	
Fructose	510	(280-770)
	500	(150-875)
	511	(352-901)
	598*	(103-931)
	160*	(26-872)
Flavin	0.05, 1.89	(0.152-0.506)
Glutamic acid	0.35	
Glycerol phosphorylcholine	350	(110-160)
Glycine	0.09*	
Histidine	0.16*	
Inositol	35	(25-16)
Iron	2*	(1.1)
Lactic acid	35	(20-50)
Magnesium	12	
Malic acid	<0.01	
Niacin (Nicotinic acid amide)	0.1*	(0.215-0.551)
Nitrogen	48	

Non Protein	756	
Total	877	(111-1169)
Pantothenic acid		(0.232-0.466)
Phenylalanine	0.16*	
Phosphorus acid soluble	33, 46	
Inorganic	9	
	17	(13-26)
	4	
(easily hydrolysable)		(3.6-4.6)
lipid	9	
total	82, 80	
Phosphorylcholine	trace	
Plasmalogen	142	
Potassium	288	(150-415)
	172	(50-337)
Pyruvic acid	4.7	
Serine	0.13*	
Sodium	109	(57-201)
	258*	(152-370)
Sorbitol		(10-36)
Sulphate	10	
Thiamine		(0.023-0.152)
	0.12 ± 0.02*	
Urea	4	
Uric acid	2.46*	(0.32-4.10)
Zinc	<0.2*	
	2.84	(2.5-3.65)

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centrifuged for 1 hour at 700 g. The average results of all the samples (mg/100 ml.) were as follows:—

Sample	Sodium	Potassium	Calcium
Whole semen	271	163	26
Plasma	290	161	28
Centrifuged packed spermatozoa	173	241	17

They observed that the differences between the concentrations occurring in sperm cells and plasma of the same ejaculate are highly significant for all the three cations. Further the same authors reported that the sodium and chloride concentration in the seminal plasma progressively rises and that of potassium and calcium decreases in the ejaculated semen during the frequent collections.

### EVALUATION OF SEMEN QUALITY

Evaluation of semen quality is necessary for assessing the potential fertility of a semen sample. Examination of the ejaculates is carried out as per the following procedure.

#### Care in Handling of Semen

The undiluted semen should not come in contact with cold surface of glass tubes. The artificial insemination equipment like artificial vagina, liners, cones, insulating bag and other material such as glass containers, slides, cover slips, pipettes etc. should be kept in a thermostatically controlled cabinet at body temperature. The semen samples should not be allowed to cool down below 25°C before dilution.

### Gross Examination of semen

#### COLOUR

Semen of the bull resembles whole milk in colour. Its white opaqueness is entirely due to the dense mass of sperm present. Varying shades of yellow is considered to be due to the presence of riboflavin. The yellow tinge gradually fades on exposure to light (Brochart, 1952). Melanin is also considered responsible for yellowish tinge of semen sample (Mukherjee, 1964).

Abnormal colour of semen denotes certain pathological conditions of genitalia. Yellowish colour may indicate the presence of pus or urine and a reddish or pinkish colour that of blood or degenerative tissue. Ejaculates having such abnormal conditions should be discarded.

#### VOLUME

The volume of semen has some relationship to the size and body weight of the bull, though it is something of an individual trait and even may be the family characteristic. Tomar *et al* (1965) reported a repeatability estimate of 0.431 for ejaculate volume in Haryana (Zebu) bulls. The volume may be influenced due to improper precoital stimulation and false mounting, which may result in varying amount of accessory gland secretion. The seminal vesicles, which are the main accessory glands vary in size in different bulls. More than half the portion of the ejaculate is from these glands. Singh *et al* (1967) reported in Haryana bulls an increase in semen volume upto 6 years of age. In *Bos-taurus* bulls maximum semen volume was noticed at 6 years of age in Brown Swiss and 8 years in Friesian (Zuliani, 1957). Povlicenko (1964) observed the average volume of two successive ejaculates as 7.04 ml. at 2 years and

12.31 ml. at 7 years in *Bos-taurus*. At younger age genetic differences in semen volume are not distinct, so selection of bulls for ejaculate volume is advisable after 3 years of age (Singh *et al*, 1967).

Seasonal variations are not markedly observed in respect of volume. First two ejaculates are nearly of equal volume and thereafter, the volume goes on decreasing (Prabhu and Bhattacharya, 1951). However, if the interval of semen collection is more than 7 days, second ejaculate may yield comparatively higher volume. On depletion test when 20 successive ejaculates were obtained the semen volume decreased from 4.1 ml. in first ejaculate to 1.1 ml. in the last one (Boyd and VanDemark, 1957). Frequency of collection may have same effect on ejaculate volume. Baker *et al* (1953, 1955a) reported that there was no difference in ejaculate volume by collection either once, twice or three times, a week. Singh and Prabhu (1963) observed significantly higher volume with one collection (2 rapid ejaculates) as compared to two successive collections and four times (one ejaculation) at weekly intervals from Haryana bulls. Tomar *et al* (1968a) observed no appreciable change in the volume of ejaculate by increasing the frequency from 7 to 3 days interval however, further increase in frequency of collection at daily and at two day interval reduced the

ejaculate, where as, adult bulls may give 2-12 ml. The average volume of semen per ejaculate observed in different breeds of Indian cattle is presented in Table 48.

#### CONSISTENCY OR DENSITY

The normal bull semen may be creamy or milky in consistency depending upon the concentration of sperms as follows:

Consistency	Concentration of sperm (millions/ml.)
Thick creamy	over 2,000
Creamy	1,500-2,000
Thin creamy	1,000-1,500
Milky	500-1,000
Watery	Below 100

In certain pathological conditions of testis, epididymis and accessory glands, the consistency of the semen is affected. The semen becomes thinner, less milky and watery in the initial stage of epididymitis. Lagerlof (1931) claimed the thick viscous semen as indicative of catarrhal conditions of accessory glands. In brucellosis when there is seminal vesiculitis, large purulent flocculi may be seen in the semen.

Density is only a rough estimation and is carried out macroscopically against the normal light which is confirmed by the sedimentation of sperm on

Table 48  
SEMEN VOLUME PER EJACULATION (In ml)

Breeds	Range	Average	Authors
Hariana	0.60 — 6.20	3.16	Shukla & Bhattacharya (1949)
Hariana	2.40 — 7.00	4.21 ± 0.18	Singh (1960)
Kumauni Hill	0.30 — 4.77	2.0	Shukla & Bhattacharya (1949)
Kumauni Hill	0.50 — 4.45	2.77 ± 0.10	Singh (1960)
Sahiwal	2.50 — 7.0	3.50	Kumaran (1944)
Sahiwal	0.80 — 9.00	3.80	Shukla & Bhattacharya (1949)
Sahiwal	—	2.72 ± 0.11	Bhattacharya & Prabhu (1952)
Sahiwal	—	3.99 ± 0.39	Bhattacharya & Prabhu (1954)
Tharparkar	1.8 — 6.7	3.8	Kumaran (1951)
"	—	3.10 ± 0.07	Bhattacharya & Prabhu (1952)
"	—	3.52 ± 0.09	Bhattacharya & Prabhu (1954)
Nagore	—	2.84 ± 0.11	Bhattacharya & Prabhu (1952)
"	—	3.86 ± 0.21	Bhattacharya & Prabhu (1954)
Ongole	3.0 — 4.5	4.1	Ayyar (1944)
Amritmahal	2.0 — 6.5	4.1	Kumaran (1939)
Gir	—	5.7 ± 1.00	Bhattacharya & Prabhu (1954)
Red Sindhi	—	4.71 ± 0.53	Bhattacharya & Prabhu (1954)
Indo European Cross	—	2.43 ± 0.19	Bhattacharya & Prabhu (1952)
" " "	—	3.39 ± 1.98	Bhattacharya & Prabhu (1954)
Khillar	—	3.11 (1st ejaculate)	Kodagali (1962)
Khillar	—	3.47 (2nd ejaculate)	"
		5.71 ± 0.36 (1st ejaculate)	Dutt (1967)
		1.76 ± 0.26 (2nd ejaculate)	"



The intermediate stages for concentrations mentioned above are described as follows: (D), D(D), DD(D) and DDD(D).

### Microscopic Examination of Semen

#### MASS ACTIVITY

When a drop of normal bull semen immediately after collection is viewed under the microscope without a coverslip, one can see a mass of swirling, progressively moving sperm. The microslide with a drop of semen is mounted on a spermotherm maintained at 37°C and examined under low power. The grading of mass activity is done as follows:

- O — No activity.
- +
- ++ — Wave formation with slight whirls which moves slowly across the field.
- +++ — Rapid and Vigorous waves with whirls or eddies which change with great rapidity.
- ++++ — Extremely rapid movement and churning of swirls and eddies.

advocated by Herman and Swanson (1941).

Table 49

#### MOTILITY SCORING OF SPERMATOZOA

Grado	Movement of spermatozoa	Percentage of motile Spermatozoa (Approx.)
0.	Nil ... ..	Nil
0.5	Very weak ... ..	10
1.0	Poor mostly weak and oscillatory. ... ..	20
1.5	Rapid. ... ..	30
2.0	Fair, rapid and vigorous. ... ..	40
2.5	Rapid, vigorous and progressive. ... ..	50
3.0	Good, very rapid, vigorous and progressive	60
3.5	Most vigorous, progressive & active ...	70
4.0	Very good, most vigorous with progressive swirling activity.	80
4.5	Highly vigorous and progressive with slight waves and full activity	90
5.0	Excellent highly vigorous, progressive and waves in all directions (eddies)	100

scopy for the assessment of motility of semen.

Lasely and Bogart (1943), Swanson and Herman (1944), Chang (1949) and Cupps *et al* (1953) have shown that there is no clear evidence of correlation between initial motility and fertility. However, Erb *et al* (1950), Stone *et al* (1950) and Bishop *et al* (1954) observed that the initial motility is more closely correlated with other semen characteristics than with fertility.

Bosselaar and Spronk (1952) used a photoelectric cell and a counter to estimate the activity and density of semen. Rothschild (1948) devised an electrical apparatus which measures the rate of impedance in semen. Bishop *et al* (1954) found significant correlation between the measurements of impedance change frequency and fertility.

The average initial motility of Hariana bull semen was + 3.5 and the repeatability was 0.55 (Tomar *et al*, 1965). Initial motility varies with the frequency of semen collection. If the interval of collection is more than one week, the second ejaculate is better in initial motility. However, there was no difference in motility if collections are obtained between 3 to 7 days interval. If semen samples are collected for successive days for longer period the motility is reduced to a great extent (Sayed and Oloufa, 1957; Tomar *et al*, 1968a). On rapid successive collections of semen, initial motility declines after second ejaculation (Prabhu and Sharma, 1953; Hafez and Darwish, 1956; Tomar *et al*, 1968a).

Season has got some effect on the initial motility of semen. Spring season is supposed to be the best season for initial motility followed by summer season in Zebu bulls. Humid and hot climate is harmful for the production of highly

motile semen (Swanson and Herman, 1944; Mukherjee and Bhattacharya, 1952; Kushwaha *et al*, 1955; Oloufa *et al*, 1959; Sen Gupta *et al*, 1963; Tomar *et al*, 1966).

Young bulls comparatively produce less motile semen. Initial motility increased with the age of bulls (Dimitriev, 1964; Malmberg, 1965). Lindley *et al* (1959) observed highly significant correlation between initial motility and age of the bull.

#### SPERM CONCENTRATION

The concentration of spermatozoa in an ejaculate can be determined by the following four methods (1) Direct sperm count of diluted semen by the use of haemocytometer. (2) Use of Nephelometer for comparing the light absorbing capacity of diluted semen with that of semen whose concentration has been established by haemocytometer. (3) Comparison of the density of semen with barium sulphate or other density standards calibrated against a direct count by haemocytometer. (4) Comparison of the cell volume of the semen ejaculate after centrifugation against a direct count by haemocytometer.

#### Procedure for Direct sperm count method

The haemocytometer which is used for red blood cell count is also used for sperm count. The semen sample immediately after collection should be kept in a water bath maintained at 30°C. The semen should be drawn into the micropipette (0.1 ml. capacity) carefully upto the mark. The exterior of the pipette should be wiped with a filter paper or wet cotton wool and the semen should be mixed with 9.9 ml. of physiological saline solution to make the dilution

1:100. After thoroughly mixing take 1 ml. of diluted semen in a 1 ml. graduated pipette and add to 9.0 ml. of 0.05% Eosin solution to make the final dilution 1:1000. Transfer this diluted semen sample to the ice chamber to kill the spermatozoa by cold shock.

More satisfactory results were reported by Hukeri (1969) by preparing a diluting fluid for further dilution of semen to 1:1000 as follows:— 3% sodium citrate + 0.1% Eosin Yellow + 2 drops of commercial formalin in 100 ml. of distilled water. It has been shown that this diluting fluid kills all the spermatozoa immediately and hence facilitates immediate estimation of sperm concentration of the neat as well as extended and stored semen samples.

Charge the haemocytometer with the diluted semen (1:1000) for counting the spermatozoa under the low power. Count the spermatozoa in the large 64 squares of the Neubauer haemocytometer and take over-all average for the 16 squares (1 sq. mm. area). This is multiplied by  $10^7$  for the estimation of total sperm concentration (Figs. 136, 137).

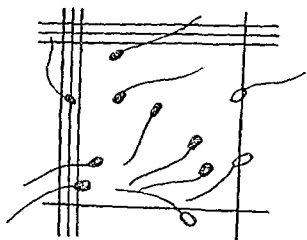


Fig. 137. Counting of spermatozoa in the haemocytometer (small square).

Sperm concentration initially depends on gonadal potentiality for spermatogenesis.

Considerable attention has been paid to the methods of estimating the density of spermatozoa. Cumming (1954) reported that there was no change in conception rate with the concentration of spermatozoa, ranging from 2.5 to  $19 \times 10^6$ /ml, though higher concentrations appear to result in higher fertility. Erb et al (1955a) found that variation in the concentration of spermatozoa was an important factor interfering with metabolic tests. The concentration increases as a result of sexual excitement caused by restraint and false mount (Collins et al, 1951; Branton et al, 1952). Hafs et al (1962) obtained significant increase in sperm output as a result of more preparation time, false mounts and interval between two single ejaculates. There is no variation in sperm density between the frequency of semen collection from 7 to 3 days interval. However, it is reduced at 18 or 21 hourly collection (Sayed and Oloufa, 1957; Tomar et al, 1968a). Singh and Prabhu (1963) observed that two rapid collections at twice a week interval yield higher sperm density than a single ejaculation at weekly interval.

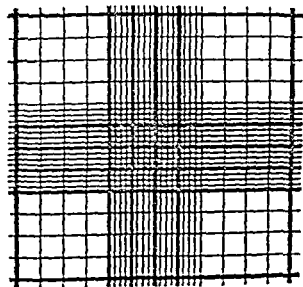


Fig. 136. Ruling of the Neubauer haemocytometer.

hypotonic to bull semen with a pH of 8.5.

Campbell *et al* (1956) stressed that the time between preparation of the subsample and the making of the smears should be kept constant as 5 minutes. The use of a citrate diluent as a solvent for the stain to reduce the number of partially stained spermatozoa, selecting the fields for counting without avoiding clumps of spermatozoa and following a definite criteria for differentiating the stained and unstained spermatozoa have also been suggested by those authors. They further showed that with a count of 20% dead spermatozoa obtained by counting a total of 400 spermatozoa (100 from one smear from each of four subsamples) the approximate variation could be  $20 \pm 6.0$  i.e. 14 to 26% where as by counting 200 spermatozoa from one smear and from each of the two subsamples, the range would be  $20.0 \pm 8.4$  i.e. 11.6 to 28.4%. Bishop and Smiles (1957) described the use of ultra violet back ground illumination alongwith premulin stain wherein dead spermatozoa fluoresce lightblue and the living ones remain invisible.

Semen samples containing initially less than 70% live spermatozoa reduced the fertility rate and are not good for preservation (Stone *et al*, 1950; Lasley, 1951; Bonadonna and Kaan, 1955) and with less than 50% live spermatozoa may be of doubtful fertility (Lasley and Bogart, 1913). Live percentage of spermatozoa may depend on frequency of semen collection. Second ejaculate may have more number of live spermatozoa if the collection interval is more than a week, otherwise it may not markedly vary if the semen is collected once in a week to every 18 hours. In successive ejaculates the live percentage of spermatozoa declines after second

ejaculation (Sengar and Sharma, 1965). Age of bull may not have any effect on percentage of live spermatozoa. In general, bulls over 3 years of age produce higher percentage of live spermatozoa (Singh *et al*, 1967). Tomar *et al* (1966) reported seasonal effect on live percentage as it is lower in humid hot season.

#### PERCENTAGE OF ABNORMAL SPERMATOZOA

Williams and Savage (1925) and Lagerlof (1934) studied the semen samples of a number of bulls and reported lower fertility when there were more than 17% abnormal spermatozoa. They attached great importance to the morphology of the head of the spermatozoa, the main abnormalities being either its abnormal shape or complete detachment. Anderson (1941) and Davis *et al* (1940a) made similar observations. Herman and Swanson (1941) claimed that the presence of abnormal spermatozoa upto 30% was compatible with good or poor fertility however, a high percentage of tail defects unaccompanied by abnormal heads was included in their observations. Rollinson (1951a) studied 29 infertile or sterile bulls and 11 fertile bulls used in natural mating and observed that the incidence of abnormal spermatozoa was not closely correlated with fertility. He reported that with an incidence of approximately 4% abnormal heads, 5% detached heads, 10% depressed midpieces and 1% tails, a fertility rate of 60% to 80% or 1.7 to 1.25 services per conception was expected.

#### CLASSIFICATION OF ABNORMAL SPERMATOZOA

The following is the classification of abnormal forms of spermatozoa based on the methods of Williams and Savage (1925), Lagerlof (1936) and Blom (1950).

(A) *Primary abnormalities* (due to defective spermatogenesis) pyriform head tapering head narrow head dwarf head giant head short thick head other abnormal shaped heads loose abnormal head normal heads with highly coiled tails double forms of head or tail abaxially attached middle piece abnormalities of the form of the middle piece spermatozoa with proximally placed protoplasmic droplets

(B) *Secondary abnormalities* (due to degenerative changes) detached normal head spermatozoa with distally placed protoplasmic droplet spermatozoa with bent tail spermatozoa in which the galera capitis has become detached

Blom (1948) reported an approximately 5 to 20% primary abnormality in a study of 100 normal fertile bulls. Secondary abnormalities varied in the incidence. He also considered that the incidence of over 3% spermatozoa with proximal protoplasmic droplets is of some significance. Bishop *et al* (1951) observed 6.1% as the mean incidence of abnormal spermatozoa in a study of 76 bulls. The classification of sperm abnormalities included the cytoplasmic droplets attached at the neck in the primary forms similar to the earlier classification described by Lagerlof (1936) and they did not include the tail deformities in the secondary form but classified as tertiary forms. They further reported 1.3%, 1.8% and 19.5% respectively as the mean incidence of primary, secondary and tertiary forms. Cupps *et al* (1953) observed a high correlation between percentage of abnormal spermatozoa and fertility. Morphological abnormalities of spermatozoa should be carried out as there are definite spermatozoal abnormalities associated with infertility. Leunissen (1946), Blom (1948), Hancock (1949)

and Rollinson and Makinson (1949) described wide recognised acrosome defect of spermatozoa of Friesian bulls. Hancock and Rollinson (1949) and Haq (1949) claimed that the defect of detached head was associated with sterility in Guernsey bull. Hancock (1955) demonstrated that the separation of head and tail of sperm occurred in caput epididymis. Cork screw type of mid piece defects reported by Blom (1959) appears to be associated with reduced fertility. Wu and McKenzie (1955) by using electron microscope reported that the acrosome cap and galercapitis were two different structures. Karras (1958) however suggested that the galercapitis consisted of an inner and outer cap. It may be emphasised here that great care should be taken in the collection, handling, smearing and staining of semen to avoid damage to the spermatozoa which may increase the percentage of abnormalities. Mercier and Salisbury (1947) suggested making thin smears on clean slides in order to reduce the artefacts. Hancock (1952) observed detached galercapitis only in dead spermatozoa which may be attributed to temperature shock. Less evident changes in the spermatozoa could be more easily detected without staining under phase contrast microscope. Jones (1962) observed thickening of mid piece anteriorly and coiled spermatozoa to have hereditary basis in Guernsey breed. Donald and Hancock (1953) noticed characteristic knobbed head of spermatozoa in Holstein Friesian bulls and they were detected due to sex limited recessive gene. Bonidonna (1955) observed knobbed heads of spermatozoa under electron microscope in Friesian bulls. Drevious (1963) observed spiralization of spermatozoa tails due to hypotonic media.

Johnston and Branton (1953) observed highest percentage of abnormal spermatozoa in fall season (ambient temperature maximum 79.9°F, minimum 55.9°F and vapour pressure 14.6 mm Hg). In Indian Zebu bulls, Mukherjee and Bhattacharya (1952) reported seasonal changes while Tomar *et al* (1966) observed no such abnormal variation in live sperm.

On account of successive ejaculates, first two rapid ejaculates generally do not vary in the percentage of abnormal spermatozoa but further collections increase the incidence of abnormality (Prabhu and Bhattacharya, 1951; Hafez and Darwish, 1956; Sengar and Sharma, 1965; Tomar *et al*, 1968 b). It has been observed that frequency of collection at 48 to 72 hours from Zebu bulls lower the percentage of abnormality (Tomar *et al*, 1968c).

Malmberg (1965) observed a decrease in the proportion of abnormal sperms as the age of bull increases.

Strikwerda (1951) observed 35.9% fertility rate in Friesian bulls with sperm having abaxial attachment compared to 57.1% with normal semen. Similar observations have been made by Raja and Nambiar (1962) in a Red Sindhi bull.

Salisbury and Mercier (1945) found that a count of 100 spermatozoa on each of the two slides from one ejaculate was as reliable as the examination of 500 spermatozoa (Fig. 138).

#### New Classification of the Bull Spermogram (Blom, 1971) :

##### MAJOR SPERM DEFECTS (1-15)

1. Underdeveloped
2. Double forms
3. Acrosome defect ('knobbed sp')
4. Decapitated sp. defect (Guernsey)

5. 'Diadem defect' (Crf 'pouch formation')
6. Pear shaped head
7. Narrow at the base
8. Abnormal contour
9. Small abnormal heads
10. Free pathological heads
11. Conkscrew defect
12. Other middle piece defects (incl. tail stump)
13. Proximal droplets
14. Pseudo-droplets
15. Strongly folded or coiled tail ('Dag-defect')

##### MINOR SPERM DEFECTS (16-24)

16. Narrow heads
17. Small, normal heads
18. Giant and short broad heads
19. Free heads (normal)
20. Detached acrosomal caps (India ink)
21. Abaxial implantation
22. Distal droplets
23. Simple bent or coiled tail
24. Terminally coiled tail

[Figs. 139, 140, 141, 142, 143, 144, 145, 146(a), 146(b), 147].

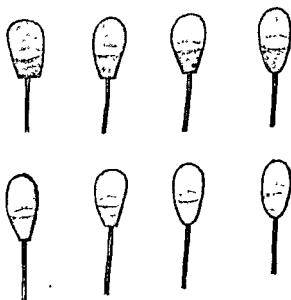


Fig. 139. Different types of Normal sperm heads.

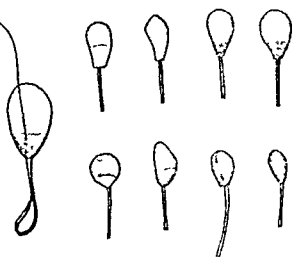


Fig 140 Giant and Dwarf sperm heads

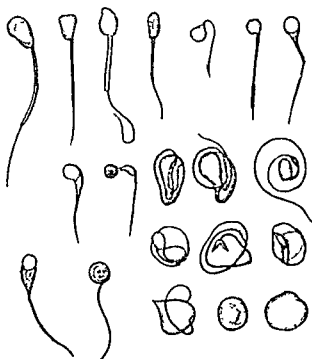


Fig 142 Under deve'oped sperm heads.

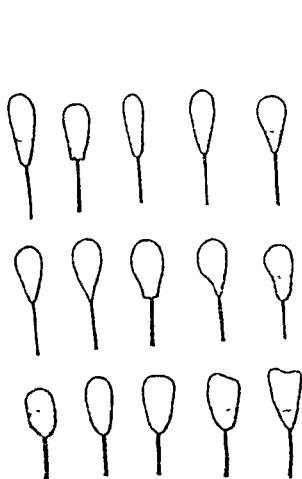


Fig 141 Upper row  
Middle row  
Lower row

Narrow sperm heads.  
Pyruiform sperm heads.  
Other pathological sperm heads.

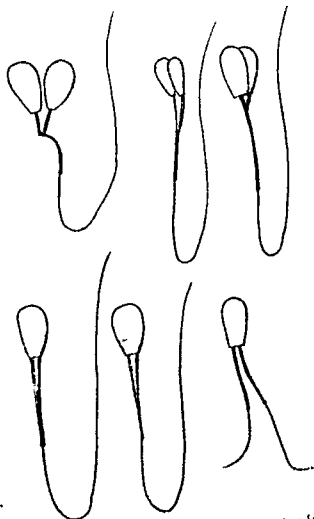


Fig. 143. Sperm with double sperm heads and pieces of tails.

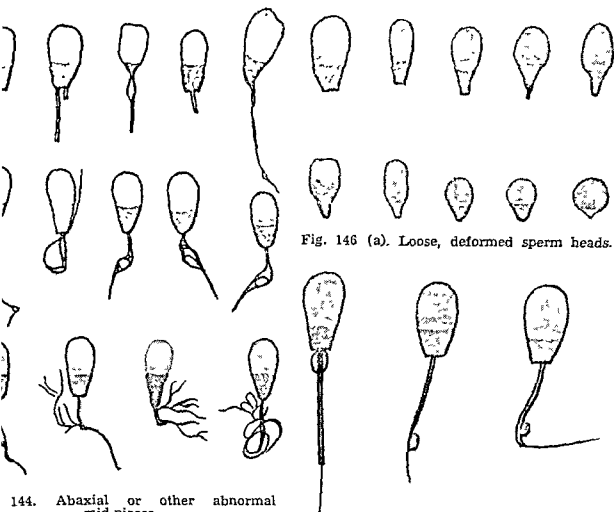


Fig. 146 (a). Loose, deformed sperm heads.

Fig. 146 (b). Protoplasmic droplets- proximal, distal.

144. Abaxial or other abnormal mid-pieces.

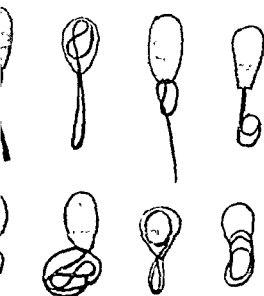
#### OTHER CELLS IN SEMEN

Other cells found in semen are medusa formations; spermiogenic cells, giant cells, preputial cells, leucocytes, erythrocytes (Fig. 148).

Certain morphological defects of Spermatozoa probably of hereditary origin are mentioned below:—

#### A. Defects of sperm head

(i) *Disintegration of bull spermatozoa*: This is a seminal defect characterised by virtual absence of intact spermatozoa observed in Guernsey breed. The ejaculate contains only free head and tails and the intact spermatozoa never exceed 5 per cent. The sexual be-



145. Sperm with bent or coiled tails.



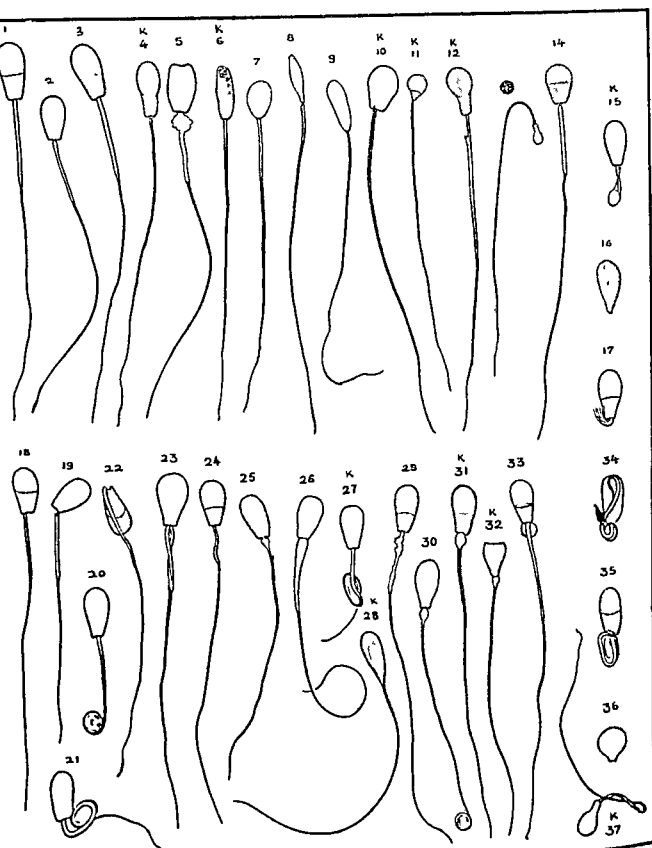


Fig. 147. Magnification:  $\times 1335$ . Staining — Eosin Nigrosin.

## Description of the spermatozoa

1. Normal, dead.
2. Normal, Live.
3. Normal, partially stained, dead.
- 4. Narrow head, dead.
5. Broad head; acrosomal abnormality, distorted protoplasmic droplet, Live.
- 6. Irregularly shaped head with dark spots, dead.
7. Round head with white spot, dead.
8. Spindle shaped head with white irregular spots, dead.
9. Dirty white coloured, narrow head with bright central longitudinal line, narrow mid piece, dead.
- 10. Pear shaped head, Live.
- 11. Micro head, dead.
- 12. Pear shaped head with torn mid piece, dead.
13. Pear shaped, micro head, dead.
14. Short head, dead.
- 15. Tightly coiled tail involving mid piece.
16. Detached head, narrow at base, dead.
17. Torn mid piece (exposing fibres), detached tail, Live.
18. Abaxial attachment, dead.
19. Broken neck, dead.
20. Terminal tightly coiled tail, Live.
21. Tightly coiled mid piece and tail, Live.
22. Broken neck, dead.
23. Double mid piece and tail with mega head.
24. Short kinky mid piece, dead.
25. Torn mid piece, proximal protoplasmic droplet, dead.
26. Swollen mid piece, Live.
- K — 27. Double coiled tail, Live.
- K — 28. Head narrow at base, dead.
29. Distorted mid piece, dead.
30. Proximal protoplasmic droplet, coiled tail at the tip, Live.
- K — 31. Proximal protoplasmic droplet, partially stained, dead.
- K — 32. Detached Galea capitis, proximal protoplasmic droplet, dead.
33. Proximal protoplasmic droplet with mid piece seen running through, dead.
34. Immature sperm, Live.
35. Coiled body and tail below head, dead.
36. Detached round head with light border at apex, dead.
- K — 37. Small pear shaped head with twisted tail, dead.

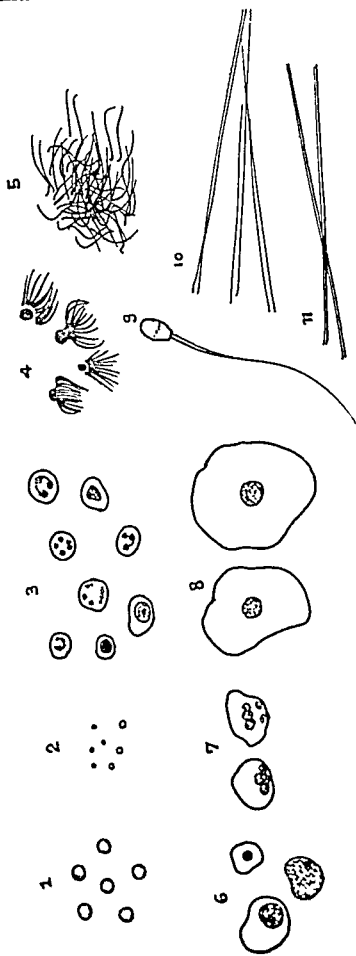


Fig. 148. Other cells in semen:—

1. Erythrocytes.
2. Free protoplasmic droplets.
3. Leukocytes.
4. Medusa formations.
5. Degenerating cluster of sperm cells.
6. Primordial spermatogenic cells.
7. Giant cells.
8. Epithelial cell casts.
9. Spermatozoan.
10. & 11. Free Sperm Tails.

lity but the concentration and morphology is normal. No detectable abnormalities are found in the testis and epididymis (Blom, 1968).

(iii) *Tail defects:*

*Dag-defect:* This is characterized by low sperm motility and high percentage of abnormal tails. The spermatozoal heads are normal while the tails are strongly coiled or folded. This defect is seen in about 40-50 per cent of the sperms (Blom, 1966).

**Staining of semen smears**

Williams and Savage (1925) and Lagerlof (1936) were the first to focus attention on the morphological structure of the spermatozoa by staining techniques. The object was to correlate the morphological difference of spermatozoa with a group of sires with varying levels of fertility. Several staining methods are in use.

8. Wash in water
9. Counterstain 1-5 sec. in methylene-blue. If stained too long with methylene — blue the smear will be decolourised. This counterstain is included in the Williams's method, but now a days it is not used.

**Staining solutions**

**STOCK FUCHSIN**

Fuchsin 10 g  
Alcohol 96% 100 ml.

**PHENOL SOLUTION**

Liquid phenol 10 ml. (Phenol liquidum 90%)  
Distilled water 170 ml.

**BLUSH EOSIN SOLUTION**

Blue eosin 1 g  
Alcohol 96% 100 ml.

**Buffered Formal—Saline**

A drop of semen is added to 1 ml. of buffered formal saline. From this mixture a wet preparation is made to study the morphology, acrosome, proximal protoplasmic droplets, mid piece and tail.

**Composition**

Stock buffer solution	100 ml.
Stock NaCl solution	150 ml.
Commercial formalin	62.5 ml.
Water	to 500 ml.

**Stock Buffer Solution**

$\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$	21 682 g )	200 ml.
$\text{H}_2\text{O}$	to 500 ml. )	
$\text{KH}_2\text{PO}_4$	22 251 g )	80 ml.
$\text{H}_2\text{O}$	to 500 ml. )	

**Stock Sodium Chloride solution**

NaCl	9.10 g
$\text{H}_2\text{O}$	to 500 ml.

Lagerlof (1936) has also recommended heated and cooled opal blue solution for staining the back ground of semen smear. By this the spermatozoa are not stained but the cellular outline is clearly visible. Lawley *et al* (1942) used opal blue solution with Eosin as the back ground stain in vital staining technique. Eosin Nigrosin stain and Giemsa stain are also commonly used.

**Biochemical tests**

The metabolism of semen is predominantly glycolytic as suggested by Mann (1949). A correlation between the metabolism of semen and its fertilizing capacity is tried to be established by the extensive study on the respiratory mechanism and dehydrogenase activity of semen.

**Hydrogen-ion-concentration (pH of semen)**

The nature of anaerobic metabolism of spermatozoa leads to acid formation and produces variation in the pH of the diluent. This depends on the degree of the metabolic activity, availability of carbohydrate substrates and the buffering capacity of the medium. The pH is estimated by use of the Capillator (BDH) with the bromothymol blue or bromocresol purple indicators. Precise estimation is always done by the use of electro pH meter. (Table 51).

Laing (1915) recorded low fertility in bulls having initial pH above 6.7. Highly motile semen is acidic whereas poor motility and low concentration of spermatozoa leads to alkaline semen. There was no significant difference in pH of first and second ejaculates in exotic and Zebu bulls (Wussow and Schroder, 1962) but it has been found to increase progressively in successive ejaculates (Tomar *et al*, 1968 a). Season has effect on initial pH in the semen of exotic and Zebu bulls (Swanson and Herman, 1944; Mukherjee and Bhattacharya, 1952). The pH of semen is found higher in hot and humid season when initial motility and sperm density are comparatively lower (Tomar *et al*, 1966).

**Methylene Blue Reduction Test**

Property of change of colour on addition of hydrogen ion is widely used by biologists in studies of cellular metabolism. When two hydrogen ions are added to methylene blue it loses the deep blue colour and reduces to leuco methylene blue. The reduction time taken by the methylene blue depends on the dehydrogenase activity of the semen and is assessed by estimating the time required by a given semen sample to

Table 51  
pH OF SEMEN IN VARIOUS BREEDS OF COW-BULLS

Breeds	pH (initial)	Reference
Hariana	6.7	Tomar <i>et al</i> (1966)
<i>Bos taurus</i>	6.73 6.69	Anderson (1942) Wussow and Schroder (1962)
Khillar	6.3 (Cold season) 6.4 (Wet season) 6.6 (Hot season)	} Kodagali (1962)
Khillar	6.1 $\pm$ 0.05 (1st ejaculate) 6.52 $\pm$ 0.06 (2nd ejaculate)	

de-colourise methylene blue solution under standard conditions of incubation. Bishop *et al* (1954) in 112 observations on 76 bulls found an average reduction time of 7.5 minutes with semen diluted in egg yolk phosphate buffer; 3.3 minutes in samples with insufficient fructose and 6.6 minutes in the presence of sufficient fructose. Fib *et al* (1950) and Bishop *et al* (1954) suggested that the reduction time is directly related to the motility and sperm concentration but inversely to the percent

age of live spermatozoa. They found a significant direct relationship between fructolysis per ml. of semen and fertility but not between fructose utilization per living spermatozoa and fertility; a highly significant direct relationship was found between fructolysis per ml. of semen and the concentration of live spermatozoa.

### Hyaluronidase content

Hyaluronidase designates a mucolytic enzyme or rather a group of enzymes and are found in abundance in the mammalian testis and the sperm. It originates in the seminiferous epithelium of the mature testis and is associated with the spermatozoa and not the seminal plasma. The hyaluronidase content per sperm cell is highest in rabbit and bull, less so as in man and boar, very little in dog and practically nil in birds and reptiles. Its physiological role is still uncertain.

Johnston and Mixner (1950) observed that hyaluronidase content in semen increases many times when incubated at 37°C or stored at 5°C. This increase parallels the decrease in sperm motility. Significant different levels of hyaluronidase between the bulls was reported by Johnston *et al* (1949). Later Johnston and Mixner (1950) observed no significant relationship between its content and fertility. Sallman and Birkeland (1948) indicated that the fertility was reduced after a certain threshold level. It is rather difficult to correlate the hyaluronidase level with fertility since the sperm concentration and dilution rates are required to be taken into consideration. The addition of hyaluronidase to the semen improved fertility as reported by Kurczok *et al* (1916) and Rowlands (1944), but Chang (1917) did not find any increase in the fertility.

### Resistance of spermatozoa to environmental changes

#### EFFECT OF LOW TEMPERATURE STORAGE

Anderson (1945) has reviewed the effect of low temperature storage on undiluted semen and found no correlation between the survival rate of undiluted semen cooled slowly to 5°C and

the initial motility. However, Swanson and Herman (1944) have shown highly significant linear correlation ( $r=0.84$ ) between the survival rate of undiluted semen and fertility.

#### EFFECT OF HIGH TEMPERATURE STORAGE

High temperature as that of 39°C is destructive to spermatozoa as is generally observed in febrile conditions.

#### RESISTANCE TO COLD SHOCK

It is generally known that the spermatozoa of all species are affected by a sudden drop in temperature. Mann and Lutwak-Mann (1955) demonstrated that cold shock was characterised by a decrease in the essential adenosine triphosphate (ATP) content of spermatozoa. Loss of intracellular protein which results in the reduction of glycolysis and motility have also been reported. Lasley and Bogart (1943) demonstrated a relationship between cold shock and fertility on the survival rate of spermatozoa in the diluted semen subjected to cold shock (cooled suddenly from 30° to 5°C). The effect of cold shock is minimised by egg yolk in semen diluents owing to the presence of lecithin in the egg yolk (Kampschmidt *et al*, 1953). Lasley *et al* (1942) proposed the concept of resistance of spermatozoa to the temperature shock as an estimate of semen quality. In this experiment semen having the known concentration of spermatozoa was subjected to cold shock by immersing the test tube into ice water for 10 minutes and estimating the number of spermatozoa survived by live and dead staining method. Anderson (1945) have reviewed the effect of cooling on diluted and undiluted semen. Bishop *et al* (1954) demonstrated the effects of cold shock by using nitrogen gas as a cooling agent.

(Maule, 1962). Sodium citrate acts as a chelating agent which ties up calcium and other heavy metals and disperses the fat globules in the yolk in such a way that individual spermatozoa can be observed clearly under the microscope. This made yolk citrate diluent more popular and has become the accepted standard of comparison for all other diluents.

### Modification of the yolk citrate diluent

#### 1. QUANTITY OF EGG YOLK

There was no effect on fertility when the yolk concentration was reduced to 20% but with further reduction there was adverse effect on fertility. There was no significant difference in the fertility level of the bull semen containing varying proportions of egg yolk between 50 per cent and 25 per cent (Almquist, 1951a; Holt, 1952c; Stewart *et al*, 1950).

#### 2. WHOLE EGG CITRATE DILUENT

Even though the whole egg diluent is easy to prepare, the conception rates were lower as compared to yolk citrate diluent (Dunn *et al*, 1953a; Hendrikse and Joling, 1954). This has been also reported by Milk Marketing Board (England and Wales, 1952).

tion of glucose resulted in improved motility and had no adverse effect on fertility. Ohms and Willett (1958) on their extensive fertility trials found an increased conception rate on first day non-return with glucose yolk citrate (67.8%) than that of yolk citrate (66.0%) but on second day of storage there was a significant reduction in fertility with glucose-yolk-citrate diluent (55.7%) as compared to yolk-citrate (61.7%).

Kampschmidt *et al* (1951, 1953 b) reported the use of glucose to maintain the optimum osmotic tension, pH, maximum motility and survival ratings in the diluent consisting of one volume of egg-yolk added to five volumes of a mixture containing 1 part of a 1.3% sodium bicarbonate solution and 4 parts of a 5% glucose solution. Similar findings were noted on spermatozoal survival with variation in individual bull samples by Smith *et al* (1954), and Dimitropoulos (1954). Willett and Ohms (1957a) also



down of certain amino acids in the presence of oxygen.

Jahnel (1954) reported agglutination of spermatozoa in egg yolk citrate diluent especially when the yolk was of a pale colour due to carotene deficiency in the hen's diet. This agglutination varied in ejaculate of the same bull and occurred readily with diluents of low pH.

### Yolk Phosphate diluent

Phillips (1939) on his discovery made the first announcement of the hen's egg yolk as a diluting medium and from this time wide scale artificial insemination programme in cattle commenced in the United States. Lardy and Phillips (1939) in their pioneer work were the first to describe the yolk phosphate dilutor for preserving cattle semen which consisted of one part of fresh yolk from hen's egg and one part of phosphate buffer composed of 2.0 g. of  $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$  and 0.2 g.  $\text{KH}_2\text{PO}_4$  per 100 ml. of glass distilled water and adjusted to a pH of 6.7-6.8. They reported satisfactory conception rate from diluted semen preserved upto 180 hours at  $5^\circ\text{C}$ . Further field work on this was carried out by Willett *et al* (1940) who reported considerably higher over all conception rate of 56.6% for semen from 5 bulls used for insemination of 1548 cows during five days after collection. Disadvantage of this dilutor was that it was difficult to observe the individual motility of spermatozoa due to presence of large fat globules, otherwise Bishop and Salisbury (1955a) reported that phosphate diluent was a very suitable dilutor for preservation of semen under aerobic conditions due to its depressing action on spermatozoal metabolism and high buffering capacity which maintains the required pH during storage.

There was no difference in the conception rates of semen in yolk-phosphate and yolk-citrate diluents (Salisbury *et al*, 1941; Bratton *et al* 1949; Stewart, 1950). Campbell and Edwards (1955) reported that yolk phosphate diluent was incompatible with the antibiotic streptomycin calcium chloride salt and reduced the conception rate as compared to its presence in yolk citrate diluent. Van Dichten (1957) on his study on 7,000 first inseminations found the conception rate of 54.5% and 50.8% for yolk citrate and yolk phosphate diluents respectively. The low rate in phosphate buffer was due to being hypotonic as its freezing point depression was  $0.431^\circ\text{C}$  as compared to the optimum of  $0.55^\circ\text{C}$  and also due to an interaction between the phosphate ions and yolk.

### Yolk Citrate diluent

Salisbury *et al* (1941) were the first to invent the yolk citrate diluent which is universally accepted. They found better bull-sperm livability and good fertility results from the use of sodium citrate in place of the phosphate buffer. The diluent consisted of equal volume of fresh egg yolk and of either a 3.6% or a 2.9% solution of sodium citrate dihydrate ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ). Aschaffenburg (1950) and Salisbury *et al* (1948) found that a 2.9% citrate buffer was isotonic with bull semen. However, Melrose and Stewart (1956) did not find any difference in conception rates with equal volume of either 2.9% or 3.6% sodium citrate solution and egg yolk. Pursley and Herman (1950) found no adverse effect of fluid of different isotonicity of sodium citrate concentrations from 2.3 to 3.5% on spermatozoal morphology and survival. It is not always necessary to adjust the pH after addition of egg yolk in the already adjusted pH of sodium citrate to 6.9

(Maule, 1962). Sodium citrate acts as a chelating agent which ties up calcium and other heavy metals and disperses the fat globules in the yolk in such a way that individual spermatozoa can be observed clearly under the microscope. This made yolk citrate diluent more popular and has become the accepted standard of comparison for all other diluents.

### Modification of the yolk citrate diluent

#### 1. QUANTITY OF EGG YOLK

There was no effect on fertility when the yolk concentration was reduced to 20% but with further reduction there was adverse effect on fertility. There was no significant difference in the fertility level of the bull semen containing varying proportions of egg yolk between 50 per cent and 25 per cent (Almquist, 1951a; Holt, 1952c; Stewart *et al*, 1950).

#### 2. WHOLE EGG CITRATE DILUENT

Even though the whole egg diluent is easy to prepare, the conception rates were lower as compared to yolk citrate diluent (Dunn *et al*, 1953a; Hendrikse and Joling, 1954). This has been also reported by Milk Marketing Board (England and Wales, 1952).

#### 3. ADDITION OF CARBOHYDRATE-GLUCOSE AND FRUCTOSE WITH OR WITHOUT SODIUM BICARBONATE

Salisbury and Van Demark (1945) reported that addition of glucose or of fructose stimulated the rate of glycolysis, even though there was no shortage of glucose in the yolk diluent. Salisbury and Knodt (1947) found no improvement in fertility with the addition of 540 mg. per 100 ml. of glucose to the yolk citrate diluent with the dilution rate of  $30 \times 10^6$  spermatozoa per millilitre. Valerani (1948) found that addi-

tion of glucose resulted in improved motility and had no adverse effect on fertility. Ohms and Willett (1958) on their extensive fertility trials found an increased conception rate on first day non-return with glucose yolk citrate (67.8%) than that of yolk citrate (66.0%) but on second day of storage there was a significant reduction in fertility with glucose-yolk-citrate diluent (55.7%) as compared to yolk-citrate (61.7%).

Kampschmidt *et al* (1951, 1953 b) reported the use of glucose to maintain the optimum osmotic tension, pH, maximum motility and survival ratings in the diluent consisting of one volume of egg-yolk added to five volumes of a mixture containing 1 part of a 1.3% sodium bicarbonate solution and 4 parts of a 5% glucose solution. Similar findings were noted on spermatozoal survival with variation in individual bull samples by Smith *et al* (1954), and Dimitropoulos (1954). Willett and Ohms (1957a) also found better results in a dilution rate of 1:9 (180 millions spermatozoa per ml.) but with high dilution rates (*i.e.* less than 15 million per ml.), the survival in yolk-glucose-bicarbonate was lower than in yolk-citrate diluent. Further they found that on storage for 10 days the pH on high dilution rate was above 7.0 as compared to 6.81 of dilution rate of 1:9. They concluded that high pH of the diluent stimulated the metabolism of spermatozoa in low concentration or high dilution rate. Senegacnik (1956) reported a very high over all conception rate with Kampschmidt's diluent. Perez-Y-Perez (1954b) found that with a buffered saline diluent a 4.5% solution of fructose was superior to glucose in promoting spermatozoal survival while the reverse results were obtained by them in non-buffered saline. Sergin (1956) who concluded that fructose egg-yolk citrate diluent produced better viability of

spermatozoa and less lactic acid from the metabolism of fructose than that in glucose egg yolk citrate diluent Kolk and Van Dielen (1957) on their study involving over 16 000 inseminations did not find any beneficial effect on fertility by addition of 1.0% fructose in a diluent even though spermatozoa survival was improved

Addition of carbohydrates glucose and/or fructose has resulted an increase in the motility and survival of spermatozoa but showed no effect on increase in conception rate Maintenance of the correct osmotic tension and electrolyte balance is more important with addition of carbohydrate than its utilisation by the spermatozoa

#### Glycine-containing diluents

Knoop and Krauss (1914) made the addition of 1.09% glycine to the yolk phosphate diluents and reported better livability of spermatozoa on storage at 5°C as compared to yolk phosphate and yolk gelatine diluents Roy and Bishop (1954) recommended equal volume of egg yolk and 1% glycine solution in distilled water of the egg yolk glycine dilutor for preservation of bull semen at 5°C and observed slow decline in motility and increase of mean half life of spermatozoa by 6 days than in yolk citrate and yolk phosphate diluents Baier *et al* (1957) also found better results with egg yolk glycine while as Strom (1956) found insignificantly higher conception rates in yolk citrate than in yolk glycine after storage for 50 hours or more in a split sample trial involving 3 000 inseminations Adler and Rasbech (1956c) compared yolk citrate and glycine fructose yolk diluents used within 12 hours of collection on controlled trials with 8 000 inseminations and found conception rates of 66.2% and

66.0% respectively while as uncontrolled trials on 400 inseminations by yolk glycine diluent for 7 days at +4°C the conception rate was 27.5% Glycine diluent even though has beneficial effect on the survival of spermatozoa there is no improvement in fertility of bull semen Rikes and Stallcup (1956) recorded the pH and found that glycine does not buffer the diluted semen as compared to sodium citrate

#### Milk Diluents

Kolliker, a German investigator was first to publish in 1856 the use of milk as a bull semen diluent but his work was not widely spread since at that time there was little interest in prolonging sperm life (Thacker and Almquist 1951)

#### Whole Milk

Michajlov (1950) stated that boiled filtered milk gave highly satisfactory results at the dilution rate of 1:25 Thacker and Almquist (1953) reported that in either homogenised whole milk or skim milk the sperm cells die in a day or two unless the milk was first heated gently for a few minutes Almquist and Thacker (1952) indicated that the heated milk gave better fertility results with low fertility bulls compared to yolk citrate diluent This was later confirmed for boiled milk as compared to yolk phosphate and yolk citrate by Dreher and Webb (1953), Almquist (1954) and Perkins *et al* (1955) The data presented by Salisbury (1957) on the summary of the fertility results show that heated homogenised milk with or without antibiotics penicillin and streptomycin gave fertility results (69.1% — 70.9%) almost equal to that of yolk citrate containing the two antibiotics (67.1% — 68.4%).

## Skim Milk

Thacker and Almquist (1951) reported the use of pasteurised homogenised milk and pasteurised skim milk as better diluent fluids for bovine semen. Jacquet (1951) found 9 to 15% better conception rates by the use of canned skim milk diluent compared to egg yolk citrate. Weiss (1952) reported 58.5% and 59.3% conception rate of first inseminations of 200 and 5000 cows with egg yolk skim milk and egg yolk citrate dilutors respectively. Marion and Olson (1952) used reconstituted skim milk as a diluent and found it equally well with egg yolk citrate for bovine semen preservation. The findings of Almquist *et al* (1954), Kerruish (1956), Melrose (1956), Adler and Rasbeck (1956b) and Melrose *et al* (1958) show that heat treated fresh skim milk is as effective as yolk-citrate. However Bolton and Durrell (1954) suggested that the reduced conception rate with milk may have been due to the higher degree of bacterial contamination existing in milk in spite of its having been heated to 96°C to 100°C. Almquist *et al* (1934) reported the fertility results on 8399 first inseminations with 72 ejaculates from 8 Holstein bulls as 67.8% for heated skim milk. Seacke *et al* (1955) found heated fortified skim milk better than the fresh milk for the livability of bovine spermatozoa. Jaskowski (1956) made comparative investigations on the preservation of bull semen in egg yolk skim milk (1:9), egg yolk citrate, egg-yolk glucose-citrate and boiled skim milk. They found that there was no significant difference between the diluents and livability of spermatozoa at 1 to 10 dilution rate. At dilutions of 1:80, egg yolk skim milk was much superior (51.5%). Hendrikse and Joling (1937) reported the results of the first day se-

men as 6.2% better with the citrate compared to skim milk while as the subsequent results gave better with egg yolk skim milk dilutor. Melrose *et al* (1958) found powdered skim milk to be as effective as fresh skim milk diluent. They also have reported the increased efficiency of the antibiotic addition to skim milk diluent and also have indicated the need for using a powder from a reliable source. It is apparent from the above that the skim milk has a promising role as a semen diluent as the fertility results from the use of properly heated skim milk are as satisfactory as compared to others.

## Reconstituted non-fat dry milk solids

Melrose *et al* (1958) reported that high temperature spray dried skim milk used as 9% of a diluent with glass distilled water and streptomycin gave high fertility than that for yolk citrate with streptomycin. The reconstituted skim milk was carefully heated at 92°C for 10 minutes in a double boiler. The diluted semen was used on the first and second day of collection and the reconstituted skim milk gave superior results each day.

## Effect of raw or unheated milk on spermatozoa

Most of the commercial milk samples contain substances toxic to spermatozoa. Thacker *et al* (1951) reported that the toxic factor or factors are associated with the albumin fraction of milk protein. Flipse *et al* (1954) observed that lactenin and antistreptococcal substance of milk was highly toxic to spermatozoa. Though milk contains a wide variety of milk enzymes which may be destroyed by heating, lactenin may be primary factor responsible for the toxicity of unheated milk. Protein fractions of milk

if heated above 80°C will release sulphhydryl groups which may act as reducing agents controlling oxidative metabolism of spermatozoa. Thacker *et al* (1954) found that 6  $\beta$  lactoglobulin, a milk protein fraction which will release sulphhydryl groups on heating is not toxic before heating. However Boyd *et al* (1954) observed that thioglycolic acid when added to pasteurised milk produced the same improvements in milk, as does heating to 92°C. Similar observations were made by Johnson *et al* (1955) who concluded that the SH group (sulphhydryl) of cysteine hydrochloride acts directly on the lactenin of milk to irreversibly inhibit its toxicity. This effect is produced by the release of SH groups from milk proteins by heating. Van Demark (1952) found that seven parts of commercial pasteurised skim milk unheated before use plus three parts of 2.9% sodium citrate dihydrate 1 l yolk, preserved spermatozoa motility better when stored at 5°C and yielded a slightly higher fertility as well. The fertility results for the yolk citrate plus penicillin and streptomycin were 68.5% for 681 first services with the skim milk plus yolk citrate, penicillin and streptomycin fertility results were 70.3% for 671 first services. This observation has shown that the small amount of yolk (a total of 15%) apparently protected the spermatozoa against any toxic substances in the skim milk which was unheated before use.

### Cream

Adler and Rasbech (1956b) obtained a conception rate of 69.1% for the 2931 first inseminations by using the homogenised sterilised 9% export cream as a diluent, compared with 63.6% for 2370 first inseminations with egg yolk citrate

and 69.22% for 2263 first inseminations with heated skim milk.

### Milk Glycine and/or glycerol diluents

Flipse and Almquist (1956) observed that survival of spermatozoa in equal volumes of heated fresh skim milk and 0.5 M glycine was equal to that in yolk glycine and superior to that in heated skim milk alone. Results obtained with skim milk powder were similar and survival rates were further increased by adding either fructose (0.2%) or glycerol (5.0%).

Since Almquist (1957) claimed an extended fertilizing life for spermatozoa in semen diluted in milk plus 10% glycerol, more attention has been directed to the use of glycerol alone in milk. Ohms and Willett (1957) reported markedly reduced fertility by using glycerol in a skim milk powder diluent compared with yolk citrate. Williams *et al* (1957) demonstrated a highly significant increase in conception rate with the milk glycerol diluent on the second and third day of use. Similar observations were made by O'Connor and Smith (1959) who obtained an increase of 1.7% and 10.6% with the skim milk glycerol diluent used on the second and third day after collection respectively. Almquist (1959) emphasised that the glycerol must be added after the semen, diluted initially in milk alone has been cooled down to 5°C. He reported that the results with this procedure would increase conception rate by 3% compared to milk alone.

### Other Diluents

Other substances like Gelatin, Sodium salicylate, Sodium carbonate, Sodium chloride, Blood serum, Blood plasma, Seminal plasma, Tomato juice and

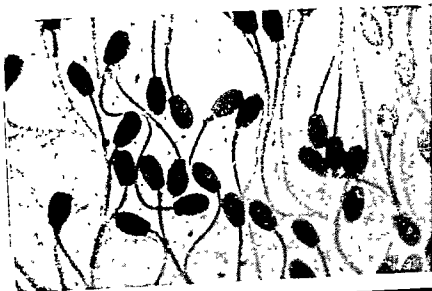


Fig. 138 Stained spermatozoa



Fig. 151. 'Fern patterns' of cervical mucus at oestrus.

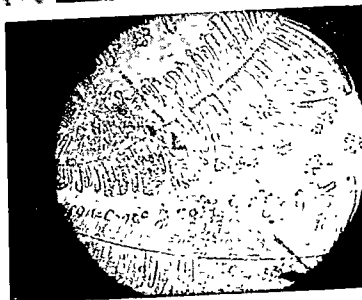


Fig. 149. Semen shippers (a) Poona model  
(b) Bangalore Model



Honey have been used as semen diluents by various workers.

### Semen additives

Addition of enzymes like mucinase, catalase, hyaluronidase and antistin as well as hormones like thyroxin, estrogen, progesterone and vitamins like vitamin B<sub>12</sub>, Vitamin E have also been tried in semen diluents. However, extensive fertility trials have not been conducted to obtain any conclusive results.

### Commercial Diluents

The object of commercial dilutors is to make the buffer solution instantaneously available for extending neat semen. Synthetic media containing freshly purified lipids, glucose, buffer, gum products have been made use of in the commercial dilutors. To these dilutors, yellow of the egg and antibiotics are freshly added (Phillips and Spitzer, 1946). SPV 161, Spermasol-N, Spermasol-S, Diloften and Seminan are the other known commercial dilutors.

### DILUENTS FOR THE PRESERVATION OF SEMEN AT ROOM TEMPERATURE

#### Coconut Milk Extender (C.M.E.)

Norman *et al* (1962) advocated the use of coconut water as diluent for preservation of semen at room temperature. The method of preparation is as follows:

To 60-70 ml. of cooled sulphanilamide solution add:

Sodium citrate dihydrate	2.16 g.
Penicillin G. Sodium	0.60 mg.
Dihydrostreptomycin sulphate	135 mg.
Polymixin B Sulphate	10 mg.
Niastin-mycostatin solution	1.5 ml.
Coconut water	17 ml.
Egg yolk	7 ml.
Catalase solution	0.5 ml.

Bring the volume to 100 ml. with distilled water, shake well and adjust the pH to 7.4. The diluted semen in vials are stored at room temperature in the dark. Norman *et al* (1968) reported 55 to 61% (based on pregnancy diagnosis) and 64.5 to 72.0% (non return) conception rates by the use of C.M.E.

### Illini Variable Temperature diluent (I.V.T.)

Van Demark and Sharma (1957) demonstrated a possible method of storage of semen at room temperature. This diluent consisted of 20.0 g. of sodium citrate dihydrate, 2.1 g. sodium bicarbonate, 0.4 g. potassium chloride, 3.0 g. glucose and 3.0 g. sulphanilamide in 1 litre of distilled water. Carbon dioxide gas is passed through the solution until the pH reaches to 6.3. Then streptomycin 1000  $\mu$  g. and penicillin 1000 I.U. per ml. were added together with egg yolk (final concentration of yolk 10%). The diluted semen is then stored in 1 ml. ampoules at room temperature. Conception rate of 75.7% was reported in preliminary trials with semen stored at room temperature upto 7 days compared with 66.9% by using semen diluted in egg yolk citrate and stored upto 3 days at 5°C. Van Demark and Bartlett (1958) suggested higher levels of glucose and egg-yolk together with the addition of catalase and storage at 40°C.

### Use of Dyes in Identifying semen

Special dyes are added to the diluted semen to reduce errors in its handling. Phillips (1945) was first to observe the wrong semen samples being used in artificial insemination practice and suggested the use of breed identifying dyes for preventing such types of errors. He recommended neutral red and Sudan III as colour additives in the diluted semen.

Almquist (1946) demonstrated that coal tar dyes of the red, green, purple and brown colours which are used as colour additives for food could also be added in egg yolk diluents. He observed that there was no decrease in the sperm livability on storage at 5°C for 10 days. Further he reported that there was no significant difference in the fertility rates of the semen sample with or without dye additives.

### BACTERIA IN SEMEN

Semen ejaculates which are completely bacteria free can not be collected in routine collection from bulls. The knowledge of microbiology of semen is essential because of (a) the possible transmission of disease causing organisms via semen to many females, (b) the possible direct or indirect effect of the infection on spermatozoa causing reduced ability to fertilize ova and (c) the affinity of bacteria for vital substances resulting in decreased livability of stored spermatozoa.

Rozsa (1950) claimed that the semen collected at regular intervals from 8 healthy bulls contained an average of 80 000 bacteria/ml, range being 200–100 000. There are conflicting reports about the effect of bacterial contamination on fertility. Bush *et al* (1950) observed a correlation between count of diluted semen and fertility. The main bacterial species reported were *Corynebacterium pyogenes*, *Pseudomonas* and *Micrococci*. Almquist *et al* (1949), Halket *et al* (1949) and Prince *et al* (1949) however could not find a relationship between either the number of types of bacteria in semen and its fertility. They demonstrated the presence of *Pseudomonas aeruginosa* in the semen of both high and low fertility bulls. The above investigations were mainly con-

cerned with bacteria generally considered as contaminants. However it is dangerous to use semen with high bacterial count.

There is enough evidence of pathogenic organisms viz. *Vibrio foetus*, *Leptospira*, PPLO and *Trichomonas* which cause spread of disease via semen. Investigation of abortions due to *Vibrio foetus* infection revealed that the bulls were the main source of infection resulting in low conception rates in the herd. Joyner and Millar (1952) drew attention to the spread of *Trichomonas foetus* infection through artificial insemination by infected semen. TePunga and Bishop (1953), Bryan (1955) and Fennestad and Borg Petersen (1958) reported that presence of *Leptospira* in bull semen was associated with abortions. Although *Pleuro pneumoniae* like (PPLO) organisms have been demonstrated by Edward *et al* (1947) in bull semen, Albertsen (1955) detected PPLO in 85% of 85 semen samples and Bakos *et al* (1959) in 10 semen samples out of 20 fertile and in 14 out of 25 low fertile bulls. They are of the opinion that PPLO did not cause genital disease. Hancock and Kelly (1948) reported the presence of *C. pyogenes* organism in the semen of 25 bulls (out of 70 bulls) examined. They considered that this organism frequently harbours within the urethra or high up in the genital tract. Bulls excreting *C. pyogenes* should be withheld from service.

### Control of bacterial infection in semen

It is possible to control the contaminants in the semen by adding the substances which are harmless or even beneficial to the spermatozoa and must also have bacteriostatic and bacteriocidal action. Substances having these proper-



ties are chemo-therapeutic compounds and the antibiotics.

#### CHEMOTHERAPEUTIC COMPOUNDS

Chemotherapeutic agents have been extensively tried to combat against bacterial contaminants in the semen samples. However, no beneficial results have been accrued to reduce bacterial toxicity and prolong sperm life.

#### ANTIBIOTICS

It is recommended that the addition of 500 to 1000 i.u. of penicillin G sodium (crystalline) and 500 to 1000 micrograms Dihydro streptomycin sulphate/ml. of diluted semen is effective to control the bacterial contamination.

Antibiotics of oxytetracycline group, chlorotetracycline and chloromycetin, neomycin and polymyxins have also been tried for controlling bacterial contamination in the diluted semen with varying results.

It is highly advisable to use antibiotics to guard against bacterial contaminants in the semen extenders as a routine practice. However, one has to guard against bulls of low fertility as they might give deceptive picture by showing slightly higher fertility and their semen preserved for longer duration.

#### Minimum Number of spermatozoa per insemination

The minimum number of spermatozoa required per insemination to obtain an optimum fertility rate, is 5 to 10 million motile cells from bulls of known fertility (Salisbury *et al*, 1945; Bratton *et al*, 1954).

#### Dilution Rate

Salisbury (1946a) claimed that with

egg yolk citrate at the dilution rate upto 1:100, no adverse effect on fertility was observed. Melrose (1952b) observed similar results even when the semen was used upto 3 days after collection. However, he reported that there was decline in conception rate from 1st to 3rd day of all dilution rates even with the addition of streptomycin. Bonadonna (1950a) obtained an improved conception rate at the dilution rate of 1:20. Salisbury and Bratton (1948) reported that there was progressive decrease in fertility when egg yolk citrate as diluent was used at the higher dilution rate over 1:100. Willett (1950) suggested that the threshold dilution level may vary between bulls, in some as low as 1:80 but in general in the dilution rate above 1:100 level, the decline in fertility bore a curvilinear relationship in the number of spermatozoa inseminated. He further observed with the decrease in number of spermatozoa that there was 0.5% reduction in conception rate for each million fewer spermatozoa until below 6 million per insemination. The conception rate further abruptly declined from 2 to 6%, per million decrease in spermatozoa. Rottensten and Anderson (1956) used dilution rates from 1:15 to 1:47 and found that the number of spermatozoa inseminated did not affect the conception rate but the length of storage of semen had adverse effect. Bratton *et al* (1954a) reported a relationship between the number of motile spermatozoa per insemination and conception rate. They observed 70.9% conception rate with 9.5 million motile spermatozoa and conception rate was significantly lowered to 66.7% when there were 4.7 million motile spermatozoa (i.e. at the dilution rate of 1:300).

### Cooling of Semen

Cooling of semen is done to prolong the viability and fertilizing capacity of the spermatozoa by reducing the metabolic activity to a minimum. Rapid cooling of diluted semen adversely affects the motility of spermatozoa. Foote and Bratton (1949) observed in a study of split sample involving cooling from 30 to 5°C over 75 minutes obtained a significantly higher conception rate and motility rating with the semen diluted 1:4 in yolk citrate plus sulphanimide. Several workers prefer to do the initial dilution as recommended by Anderson (1945) at 30°C.

The practice of diluting the semen wholly or partially prior to cooling has almost been universally adopted. The diluted semen vials are wrapped with cotton wool and placed in a beaker containing water which is transferred to the refrigerator for further cooling to 5°C.

### Transport of Semen

It is generally observed that the motility of the spermatozoa is adversely affected if the semen sample is subjected to vigorous agitation. Bretschneider (1936) observed that vigorous shaking of the bull semen for about 3 minutes destroyed its motility. Mann (1945a) reported that when semen sample is vigorously shaken in the presence of air, the cytochrome enzyme within the spermatozoan was oxidised. It is therefore advisable to fill up the tubes completely with semen sample in order to avoid any air space. Prince and Almquist (1948) and VanDemark *et al* (1949) reported the detrimental effect on motility of semen stored in partially filled tubes and attributed the cause to increased availability of oxygen to the spermatozoa, especially in transit when

the tubes are shaken. Dunn and Welker (1957) observed no differences in conception rate in semen transported either in glass or plastic containers and suggested on their investigations covering 23000 first inseminations that plastic containers could be used safely. Sane *et al* (1954) reported on their experience at the A.I. Centre Poona, that the effect of agitation was markedly observed on transport of semen to long distances by bus. Within about six hours the motility dropped from +5 to +3 on its delivery at the A.I. Sub-centres. The addition of liquid paraffin on the top of the diluted semen did not materially alter and not much difference was observed between those with or without addition of liquid paraffin. Nirmale (1976) reported that the transport of diluted semen in Poona type (wooden) shipper was better than the Bangalore type (metal), as the agitational effect on seminal attributes was minimal. He further stated that transport of semen in small dose vials (1.3 ml.) was beneficial as it almost maintained similar values for all the seminal attributes studied during transport from 25 to 175 km, compared to those in the stored (small dose) vials.

### METHODS OF SEMEN TRANSPORT

Bull semen can be transported to long off distances by road, sea or air. The procedure for packing and transport is as follows:

1. After collection of semen, it is examined for quality and then diluted and stored under refrigeration at 5°C.
2. Quantity of semen to be transported should be put in a sterile vial from the bulk sample. The vial should be filled in completely upto the brim and fitted with rubber or plastic cork. A

proper label should be fixed on the vial with name, number of sire and date of semen collection. Since labels may give way, marking may be done with coloured glass pencil in addition.

3. The vial may be wrapped with cotton wool and placed in another bigger test tube. The cotton wool not only acts as insulating substance but also prevents agitation due to shocks in transit. This has reduced the breakage of semen vials to a considerable extent.

4. The thermos flask in which the vials are to be placed should be cleaned properly. A pad of cotton wool should be placed inside at the bottom of the flask. Small ice cubes should be filled in and the test tubes containing semen should be properly fixed inside in between the ice cubes and pad of cotton wool may also be placed on the top and then the cork lid should be fixed tightly. The outer metal cap of the flask may then be fixed.

5. The thermos may then be placed in a semen shipper and the package is properly labelled outside.

The object of such a contrivance is to maintain the inner temperature of the flask between  $0^{\circ}\text{C}$  to  $5^{\circ}\text{C}$ . This is however dependant on the air temperature and the frequency with which the thermos flask is opened from time to time. Care should be taken that the temperature does not rise above  $5^{\circ}\text{C}$  by putting sufficient ice in thermos flask.

#### Semen shippers

Under tropical conditions where semen is required to be transported for

long distances, the shipper should be sturdy and well insulated ( $5^{\circ}\text{C} \pm 1^{\circ}\text{C}$ ). Many containers have been devised in India but the following few are found to be comparatively better.

#### POONA CONTAINER

The dimensions of the container are L-20 cm.  $\times$  W-20 cm.  $\times$  H-30 cm. and is prepared out of strong teak wood. There is locking arrangement, handle on the top and also on both sides. Inside it is lined on all four sides, top and bottom with 2.5 cm. thick foam rubber. The container can hold a three pint thermos flask. The total weight of the container together with the flask is 5 kg. The container is very sturdy and breakage of the flasks is hardly any. It is suitable for transport by all modes (Fig. 149a).

#### BANGALORE CONTAINER

This consists of an outer aluminium vessel with a handle. The inside of the vessel is lined with sponge rubber 1.25 cm. thick. Together with the thermos flask placed inside, the weight of the container is about 2.25 kg. This is only suitable for a courier (Fig. 149b).

#### MATHURA CONTAINER

A piece of felt is rolled in between the glass flasks and the metal case of the thermos flask. Foam rubber 0.6 cm. thick is put inside the glass flask. The thermos flask is then closed carefully with a cork and plastic cap. The whole material is enclosed in a casing of foam rubber material. The weight of this container is less than 2.25 kg. Due to its specific construction it withstands rough handling.

#### I.V.R.I. CONTAINER

This consists of a double walled copper can with a space in the centre for

the semen vial. The can is filled with water, corked and frozen. The vial containing semen which is wrapped in cotton wool is then placed in the central opening of the can which in turn is wrapped in a thick felt. At the top and bottom of the metal can felt padding is provided with. The whole material is placed in an insulated canvass or rexine bag. The container weighs 4.5 kg. This container is useful for short duration and quick transport.

### INSEMINATION TECHNIQUE

In the mass scale breeding programme insemination technique is an important procedure for obtaining high conception rate with quality semen from progeny tested sires. The technique entails deposition of the semen in the cervix of a cow at appropriate time during the later part of the heat period in order to give best chance for the viable spermatozoa to meet the ovum for successful fertilization.

For actual insemination a 2 ml plastic or glass syringe attached with rubber connection to a 400 cm. glass pipette having an outside diameter of 6 mm and inside diameter of 1 mm is used. The inseminating pipettes in sufficient numbers should be sterilised in instrument steriliser, dried and individually wrapped with paper and again sterilised in hot air oven. It is then packed in sterilised stainless steel containers. Similarly syringes and rubber connections should also be sterilized and stocked. Unused pipettes are required to be resterilised.

Diluted semen from 0.8 to 1.2 ml dose is sucked into the pipette. The cow to be inseminated should be well secured. The Veterinarian should clean the vulval area with dry cotton, dilate

the lips and insert the pipette into the vagina. The semen should then be lead to the midcervix and then pipette is slowly withdrawn. Care should be taken while introducing the pipette in the vaginal passage so that it does not enter the suburethral diverticulum or enter the urethra due to forceful introduction. Introduction in the blind pouch may give a deceptive feel. This is usually experienced with new inseminators.

### Speculum method

With the speculum method it is possible to dilate the vagina, locate the cervix and introduce the pipette into the cervix for deposition of semen. A very great care is necessary in sterilizing the speculums and it goes difficult if mass scale inseminations have to be done. Number of speculums of various sizes are necessary and it is usually found difficult to have them effectively sterilized during the field work. Various types of speculums are used for dilatation of vagina in the cow. Plastic ones with built in light are preferred but these can not be sterilized satisfactorily. The Palsson type round tubular, stainless steel one available in different sizes is also useful but introduction of such a type into the vaginal passage is found to be difficult owing to round opening of the tip measuring about 5 cm in diameter. Full dilatation of the vagina with this type is not possible. The cervix however can be well located. The jaw type tapering metal speculum can be introduced with ease and on insertion it is possible to dilate the vagina. This type of speculum is popularly used.

The cow to be inseminated should be well secured since she is likely to struggle during introduction of the speculum. The tail should be properly secured.

ed on one side. A sterilised speculum is lubricated preferably with liquid paraffin. The operator on dilating the vulvar lips introduces the speculum into the vaginal passage and on dilatation locates the cervix by means of the head light torch. Insemination pipette containing 0.8 to 1.2 ml. of diluted semen is then introduced through the vaginal passage and inserted half way into the cervix where semen is syringed. The pipette and the speculum are then withdrawn. Care should be taken to avoid any injury to the vaginal walls due to manipulation of the speculum.

#### **Recto-vaginal or cervical fixation method**

This method is easy for operation and is widely used in practice all over the world. It is possible by this method to carry out intra uterine inseminations which is not possible by the speculum method. The technique is as under:

1. The operator should wear protective clothing viz. rubber gloves, smock and gumboots
2. The insemination pipette attach-

and back raked if necessary taking care that the ballooning is not caused by outside air rushing into the rectum or by irritation due to hand manipulation.

7. The operator should then try to locate the cervix and palpate the entire genitalia. Utmost care however, should be taken that the pressure induced on palpation does not lead to rupture of the Graafian follicle. The operator should roll the uterus and palpate both the horns thoroughly from apex to cervix to make sure that there is no early pregnancy. In adult cows it is quite likely to find disparity of horns. Certain amount of fluctuation due to oestrus discharge and turgidity can also be felt. The size of cervix will vary to a great extent and is dependant on the number of previous calvings. In milch breeds like Gir, Sindhu and Sahiwal under Indian conditions, after 2-3 calvings the cervix assumes a very large size and is

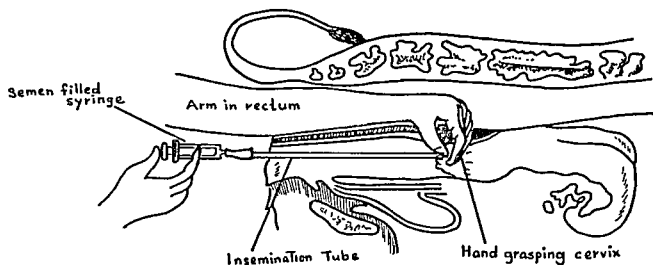


Fig. 150. Insemination technique (Recto-vaginal).

further substantiated by the fact that cervical inseminations have given higher conception rates than deposition of semen in other part of the genitalia. This may be due to the fact that cervical mucus arrests the possibility of ascending infection.

In rare instances cows may repeat to service in early pregnancy (gestational oestrus) If proper diagnosis is not made, the mid cervical insemination may save such cases from likely abortions since the pipette is not inserted in the uterine cavity.

#### QUANTITY OF SEMEN PER INSEMINATION

One ml of diluted semen is usually used per insemination by rectovaginal method and in general it is found to give satisfactory results. Olds *et al* (1953) observed no significant difference in the conception rates with either 0.25, 0.5, 1.0 or 2.0 ml. dose of diluted semen containing 24 million or 16 million spermatozoa per ml. Similar observations were reported by Lasley and Bogart (1913) following the use of either 0.5 ml. or 1.0 ml. of semen of either diluted or undiluted.

#### SITE OF DEPOSITION

Difficulty is usually experienced in inseminating heifers since the insertion of pipette in the cervix is not easy. This difficulty is particularly experienced in small statured heifers and hilly cattle where conception rate by artificial insemination is low.

Olds and Seath (1954) experienced considerable difficulty in penetrating the cervix in heifers for insemination in 11.7% of 1711 heifers resulting in the conception rate of 54% only. Similar observations were made in non-descript heifers by Sane *et al* (1954). Salisbury and VanDemark (1951) reported conception rates of 64%, 65% and 64.8% with inseminations done at the cervix, uterine body and uterine horn respectively. Pounden *et al* (1947) suggested a possibility of developing endometritis due to intrauterine insemination. Rowson *et al* (1953) demonstrated that endometritis may result from interference with the uterus during its luteal phase of the oestrous cycle. Inseminations during luteal phase may therefore prove harmful. It appears from the available evidence that there is certain amount of risk involved in intrauterine

inseminations and as such the popular view held is in favour of midcervical insemination. In order that the technician should acquire necessary skill in inseminating at the midcervix, training should be given at the abattoir by using solutions containing dyes. A beginner may pass the catheter in the shallow posterior portion close to os and if insemination is done at this site, it is as good as vaginal insemination.

clinically. It is advisable that the stock should be checked carefully for heats before they are let out from the stables during morning hours. It should be observed while in the paddocks with a teaser or breeding bull. Rechecking during afternoon and evening hours is necessary.

## 2. Crystallisation pattern of cervical

considerable importance in the economics of livestock production.

Trimberger and Davis (1943) from their studies on 295 dairy cows and heifers inseminated at various stages of oestrus observed the following conception rates from single inseminations.

Beginning of heat	44%
Middle of heat	82.5%
End of heat	75%
6 hours after heat	62.5%
12 hours after heat	28%
24 hours after heat	12%
36 hours after heat	8%
48 hours after heat	nil

On critical studies in determining ovulation through rectal palpation of the ovaries every two hours, Trimberger (1948) recorded his observations related to conception rate, as under:

Insemination time interval	Conception rate
1. More than 24 hours before ovulation ...	53%
2. 6 — 24 hours before ovulation ...	79%
3. 6 hours or less before ovulation ...	57%
4. 12 hours or less after ovulation ...	32%

There is wide range in the heat period of cows from 3-28 hours, average being 18 hours. Individual variation with regard to the length of oestrus have been observed depending on the genetic factors, environmental conditions and plane of nutrition. Proximity of bull also has certain influence on the dura-

tion of oestrus. Majority of the cows will show normal heat periods during natural breeding season than in other seasons of the year when the duration of oestrus is comparatively shorter.

Question arises whether more than one insemination is advisable during one heat period. In good herds with normal fertility this is not necessary. Repeated inseminations in the same oestrus period may show some promise only in such cows which have prolonged duration of oestrus. For mass scale application of insemination programme this is neither possible nor economical.

Cows showing abnormal type of oestrus discharge should not be inseminated. Post oestrus bleeding is observed in the continental breeds and this is of rare occurrence in the Indian breeds of cows. Autrup and Rasbech (1951) inseminated 243 cows in the haemorrhagic period about 48 hours after the end of heat resulting in the conception of 87 cows (29.69%). This low conception rate is believed to be due to either delayed ovulation or senile ovum.

Cows detected in heat during morning hours should be inseminated during afternoon hours on the same day. Those coming on heat during afternoon or the evening hours should be inseminated next morning. Such a practice results in fairly good conception rate.

Dyrendahl (1945) and Schlindler *et al* (1957) studied the effect of suspending inseminations on Sundays and holidays on the overall conception and it was estimated that by not carrying the inseminations on Sunday and postponing them over to Monday resulted in a net drop of 8.0% conception rate during season.



### **Effect of handling the cow during insemination**

Any condition such as excitement which is likely to lead to the release of adrenalin may inhibit sperm travel within the genital tract (VanDemark and Hays, 1955). Pounden and Firebaugh (1956) found lower conception rate in cows which were either completely quiet or highly nervous.

### **Optimum time for insemination after calving**

After parturition it takes about two months time for complete involution of the uterus. If heats are expressed during the period it results in lower conception rate.

ly to give higher conception rates especially in cows in which there is delayed ovulation, since their survival in the uterine tract is longer as compared to spermatozoa from bulls of low fertility.

One should not refrain from inseminating cows having irregular oestrous cycles, just on the grounds that it lowers overall conception rate. There is every chance of few of them conceiving to the service and as such insemination should not be refused unless otherwise one is certain about some pathological condition. Very little can be done for cases of hormonal imbalance.

### **BEST TIME FOR SERVICE TO OBTAIN MAXIMUM FERTILITY IN DOMESTIC ANIMALS**

mal oestrus the spermatozoa can remain viable in the uterus until ovulation. In the continental animals it is reported that during spring and summer the oestrus periods are of a longer duration. Under such conditions, if service occurs early during the oestrus, spermatozoa may not remain viable until ovulation. The real difficulty is therefore faced if breeding is by artificial insemination alone. In the controlled herds higher fertility rate is claimed when the bull is running free with the herd as compared to conception rates obtained by service through artificial insemination.

### Mare

In the mare higher fertility rates are obtained when mating occurs about 24 hours before the end of the oestrus. Natural service or insemination performed after the end of oestrus results in very poor conception rate. The duration of oestrus in the mare is very variable and as such unless a teaser is shown detection goes difficult. It is therefore advisable that the mare should be shown to the teaser every day. If the oestrus is long more than one service would be necessary to ensure that the mare is covered within 18 to 72 hours.

### Ewe

In ewes high fertility results if the services occur during the latter half of the oestrous period. When ram is running free with the herd and seasonal conditions are conducive, high fertility is usually claimed due to services in the second half of the oestrous period. On free range there is also a chance of repeated services occurring during the same period and as such most of the services are placed close to ovulation. It goes difficult with breeding by Artificial Insemination to judge the exact stage of

oestrus. There is every chance of poor conception rates in the absence of proper diagnosis as insemination may be too early or too late.

### Sow

The duration of heat in sows is on an average for 2 to 3 days and high fertility rates are claimed for services that occur before the middle of oestrus. The fertility in the sow is on the decline after the middle of oestrus and less number of ova are fertilized by mating after mid oestrus. It is usually the practice to do matings twice during the oestrous period first at about 12 hours and the second between 32 to 36 hours from the onset of oestrus. This results in the fertilization of maximum number of ova.

### Bitch

In the bitch higher conception rates are claimed if services occur during the early part of oestrus. On cessation of bleeding at the end of proestrus period when the bitch first becomes receptive to the male and if service occurs at this time, it results in high fertility rate. Fertility declines rapidly from 5th day onwards from the onset of oestrus.

### Cats

In cats, high fertility is obtained when mating occurs between 6th and 8th day after the onset of oestrus.

## RECORDING SYSTEM IN A.I.

Systematic recording in the Artificial Insemination organisation can not be over emphasized. All the information available at the Artificial Insemination organisation should be arranged in such an order that this can readily be found. By proper record keeping it is possible



1. The data regarding collection, evaluation and preservation of semen.
2. Pregnancy results obtained with semen from different bulls.
3. Individual females inseminated and the different operations carried on each of them together with the final result for every terminated service period.
4. The work carried out by each Veterinarian or the assistants employed at the A.I. centre and the results obtained by them.

The following registers are recommended for collection of the above data.

1. *Semen collection register*: It should be maintained in the following proforma (Proforma No. 1).
2. *Bull recording register*: It is to be maintained in the pro-

for individual bulls. For the inseminations carried out with each ejaculation should be entered. The name of the owner of inseminated animal including the identification of the female should be recorded. Pregnancy diagnosis should be carried out and the positive or negative results should be incorporated against each insemination. This system facilitates the assessment of fertility of each bull and also indicates the efficiency of different inseminators.

3. *Field work register*: This register either can be maintained as an individual A.I. card for each female or as cattle owners A.I. card (Proforma No. 3).
4. *Insemination register*: This should be kept in the following proforma (Proforma No. 4).

#### PROFORMA NO. 2(a)

Bull No.:

Date	Name of Owner	Cow Name or no.	Age of semen (hrs).	A. I. No.	Result	Calving date
11.3.77	A	1	24	1	—	—
	B	2		1		
	C	3		3		
	D	4		2	—	—
	E	5		1	—	
12.3.77	G	6	48	1		
	H	2		2		
	I	8		1	—	
	J	9		1		
	K	10		3		

## 43

NAME of the Centre) \_\_\_\_\_ Date of birth- \_\_\_\_\_  
Year \_\_\_\_\_

[illegible]

to collect systematic information on the following points.

1. The data regarding collection, evaluation and preservation of semen.
2. Pregnancy results obtained with semen from different bulls.
3. Individual females inseminated and the different operations carried on each of them together with the final result for every terminated service period.
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forma (Proforma No. 2 and 2a). This register should be kept for individual bulls. All the inseminations carried out with each ejaculation should be entered. The name of the owner of inseminated animal including the identification of the female should be recorded. Pregnancy diagnosis should be carried out and the positive or negative results should be incorporated against each insemination. This system facilitates the assessment of fertility of each bull and also indicates the efficiency of different inseminators.

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Date	Name of Owner	Cow Name or no.	Age of semen (hrs).	A. I. No.	Result	Calving date
11.3.77	A	1	24	1	—	—
	B	2		1		
	C	3		3		
	D	4		2	—	—
	E	5		1	—	
12.3.77	G	6	43	1		
	H	2		2		
	I	8		1	—	
	J	9		1		
	K	10		3		

**PROFORMA NO 2**

**BULL RECORDING REGISTER**

(Name of the Centre)-

Date of birth-

Year-

**Sire-  
Dam**

**Breed—**

Bull No-

**Data**

No of collections

### Semen quality

### Dilution

A. I Carried out	Identity of female
------------------	--------------------

**Pregnant!**

## Result

**ಪ್ರಶ್ನೆಗಳು**

Sex of calf  
M/F

## Inseminator

to collect systematic information on the following points.

1. The data regarding collection, evaluation and preservation of semen.
2. Pregnancy results obtained with semen from different bulls.
3. Individual females inseminated and the different operations carried on each of them together with the final result for every terminated service period.
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Bull No.:

Date	Name of Owner	Cow Name or no.	Age of semen (hrs).	A. I. No.	Result	Calving date
11.3.77	A	1	24	1	—	—
	B	2		1		
	C	3		3		
	D	4		2		
	E	5		1	—	—
12.3.77	G	6	48	1		
	H	2		2		
	I	8		1	—	
	J	9		1		
	K	10		3		



**PROFORMA NO 3  
CATTLE OWNER'S CARD**

Name of the Artificial Insemination Centre —

Serial No —

Sub Centre —

COW/BUFFALO

Owner's Name —  
Address —

Class of animal —

**BEST TIME TO BREED THE COW & SHE-BUFFALO**

Hours	Too Early	Good	Excellent time to Breed Cow	Good	Excellent time to Breed buffalo	Too late
	0	6	9	12	18	24

(After the onset of oestrus)

Approaching heat (6 to 10 hrs.)

1 Smells other cows.

2 Attempts to ride other cows

3 Vulva moist red & slightly swollen

Standing heat (18 hours)

- 1 Bellows
- 2 Off feed
- 3 Reduction in milk
- 4 Nervous and excitable
- 5 Stands to be ridden
- 6 Rides other cows
- 7 Vulva swollen moist & red
- 8 Clear copious mucus discharge

After heat (10 hours)

(1) Will not stand to mounting

Instructions to the Owner 1 Don't forget to get all informations entered in this card 2 The card should accompany the animal every time it is sent for examination 3 Do not let loose the animal after insemination due to fear of service by a scrub bull 4 Watch for the next heat period and get the animal examined 2 months after insemination 5 Don't forget to hand over the card to new owner when you sell this animal

**Artificial insemination & infertility treatment**

Identity of Female		Age	Previous History	Last calving date	INSEMINATION						Calf		Remarks & Signature
Name of animal or Tattoo No	Breed				1st		2nd		3rd		Sex	Date of Birth	
					Date	Bull	Pregnancy Diagnosis	Date	Bull	Pregnancy Diagnosis	Sex	Date of Birth	



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# Chapter 40

## Artificial Insemination in Buffaloes

The domesticated buffaloes can be classified in two categories namely the swamp buffalo and the river buffalo. Both types differ in their habits and it does not serve any beneficial purpose to cross them. The domestic buffalo (*Bos bubalis*) forms an important portion of the dairy type of animals in several Asiatic countries. India, Pakistan, China and South East Asia have a very large number. A small number is also found in Egypt, Iraq, Turkey and very few in Italy, Greece and Bulgaria.

According to FAO Report (1978) the total population of buffaloes in the world is said to be 131 million of which 128.5 million are found in Asia. India holds 61.1 million of the domesticated buffaloes of the river type, the largest concentration for any single country. The predominant milch type buffalo breeds are Murrah, Mehsana and Surti.

### Behaviour of buffalo bulls

In buffaloes the sexual maturity is comparatively later than in the cattle. The buffalo bull usually starts mounting between two and three years of age. In Egyptian buffalo bulls the average age at first service is 3.7 years (El Itriby and Asker, 1957). Dutt and Bhatta

Charya (1952) observed that spermatogenesis in buffaloes begins much earlier and meiotic division of the spermatogonial cells lining the seminiferous tubules has also been observed at the age of a year. Naik (1965) from his studies on Murrah buffalo calves observed secondary spermatocytes and spermatids at the end of prepubertal stage (38th week). He reported the presence of free spermatozoa in the lumen of the seminiferous tubules at the end of circumpubertal stage (65th week) when the calf was weighing 130 kg. He further observed the presence of spermatozoa in the epididymis when the calf was 90 weeks old.

In general it is experienced that buffalo bulls can be easily trained to serve in the artificial vagina. Buffalo bulls usually mount with ease even on the anoestrus female or on the male. In the buffalo bulls the thrust at services is not so forceful as in the cow bulls (Prabhu, 1956; Sane *et al*, 1960).

Maymone (1942) and Agabelli (1956) reported that in Italy and Transcaucasia in the USSR that the buffalo bulls attain sexual maturity at about two years of age.

MacGregor (1911) observed that in domestic buffaloes by six or seven years

there is frequently a loss of potency with gradual decrease in the conception rate as the age advances but sexual desire continues until the age of twelve or more. During this period the muscular strength also decreases. However, complete senility, that is loss of muscular strength and also the sex desire does not set in till about 15 years of age. A well bred buffalo bull maintained in excellent condition can give 80—100 natural services during a year. There is no periodical mating desire or rutting season in buffalo bulls. In Italy the buffalo bulls have to be replaced after 4 to 5 years of service (Maymone, 1912). In Egypt the average useful life of buffalo bulls is  $4\frac{1}{2}$  years (El Iriby and Asker, 1957).

Sayed and Oloufa (1957) reported that semen quality in samples from buffalo bulls from whom three collections per week were obtained was lower for all characteristics than in samples from bulls from whom only one collection was obtained during a week. On many occasions bulls from whom three collections per week were obtained failed to show libido. In Egypt, about 75% of services in buffaloes fall during four months of the year as a consequence of which the bulls are used at least three times a week during the breeding season, which probably results in the poor quality of semen and low conception rate (Asker and El Iriby, 1958).

Their experience shows that by collecting semen on alternate days, bulls show great irregularity and become sluggish in a short period. This is particularly observed in Surti buffalo bulls. Seasonal trend to a certain extent is also noticed.

### Semen collection

#### COLLECTION IN THE ARTIFICIAL VAGINA

Semen from buffalo bulls can be best collected in a short artificial vagina measuring 20 to 30 cm. in length. It is very easy to handle the short artificial vagina whereby there is hardly any wastage of semen. The artificial vagina is to be prepared and collection is to be made as in the cow bull. One has to only guard the temperature and air pressure which should remain constant. Buffalo bulls are most susceptible to temperature variations.

#### COLLECTION BY MASSAGE TECHNIQUE

This is not very satisfactory technique for the collection of semen from buffalo bulls. Singh (1976) observed that collection of semen by massage technique results in poor volume, low sperm concentration and high pH as compared to ejaculates collected in artificial vagina. Similar is the experience of Sane *et al* (1970).

### Semen Characteristics



# Chapter 40

## Artificial Insemination in Buffaloes

The domesticated buffaloes can be classified in two categories namely the swamp buffalo and the river buffalo. Both types differ in their habits and it does not serve any beneficial purpose to cross them. The domestic buffalo (*Bos bubalis*) forms an important portion of the dairy type of animals in several Asiatic countries. India, Pakistan, China and South East Asia have a very large number. A small number is also found in Egypt, Iraq, Turkey and very few in Italy, Greece and Bulgaria.

According to FAO Report (1978), the total population of buffaloes in the world is said to be 131 million, of which 128.5 million are found in Asia. India holds 61.1 million of the domesticated buffaloes of the river type, the largest concentration for any single country. The predominant milch type buffalo breeds are Murrah, Mehsana and Surti.

### Behaviour of buffalo bulls

In buffaloes, the sexual maturity is comparatively later than in the cattle. The buffalo bull usually starts mounting between two and three years of age. In Egyptian buffalo bulls, the average age at first service is 3.7 years (El Itriby and Asker, 1957). Dutt and Bhatta-

charya (1952) observed that spermatogenesis in buffaloes begins much earlier and meiotic division of the spermatogonial cells lining the seminiferous tubules has also been observed at the age of a year. Naik (1965) from his studies on Murrah buffalo calves observed secondary spermatocytes and spermatids at the end of prepubertal stage (38th week). He reported the presence of free spermatozoa in the lumen of the seminiferous tubules at the end of circumpubertal stage (65th week) when the calf was weighing 130 kg. He further observed the presence of spermatozoa in the epididymis when the calf was 90 weeks old.

In general, it is experienced that buffalo bulls can be easily trained to serve in the artificial vagina. Buffalo bulls usually mount with ease even on the anoestrus female or on the male. In the buffalo bulls the thrust at services is not so forceful as in the cow bulls (Prabhu, 1956; Sane *et al*, 1960).

Maymone (1942) and Agabeili (1956) reported that in Italy and Trans caucasus in the U.S.S.R. that the buffalo bulls attain sexual maturity at about two years of age.

MacGregor (1911) observed that in domestic buffaloes by six or seven years

(1955) in their observations on Surti buffalo bull semen observed average pH of 6.6 with the range from 6.4 to 6.8. Shukla and Bhattacharya (1949) observed average pH of 24 ejaculates of one Murrah buffalo bull as  $6.23 \pm 0.03$ . Kodagali (1967) reported 6.5 as average pH of semen obtained from four Jaffri buffalo bulls.

#### MOTILITY OF SPERMATOZOA

A motility score extending from 0 to +5 is usually used for grading the motility of a semen sample. The wave motion observed in buffalo semen is slow as compared to the cow bull. Using 0 to 10 gradation Hafez and Darwish (1956) have reported average score of 7.25 for ejaculates from Egyptian buffalo bull. The average initial motility in Murrah bull semen is reported as +3 and repeatability as 0.40 (Tomar *et al*, 1965). Kushwaha *et al* (1955) observed appreciably low motility during summer months. Buffalo bulls produce better semen during winter and spring seasons (Tomar *et al*, 1966). Rao (1958) in his studies on buffalo semen from various levels of male reproductive tract, observed that in the portion of semen from cauda epididymis in the buffalo and boar, there was little or no spermatozoal activity, whereas, in the stallion and ram, active motility was noticed from samples from the same part. The difference in motility is believed to be due to the difference in the viscosity and sperm concentration.

#### SPERM TRAVEL

Mahmoud (1952) studied the speed of travel of buffalo sperm in vitro by suspending centrifuged spermatozoa in egg-yolk citrate diluent at the average speed of 1.65 mm. per minute compared to cow bull spermatozoa of 4 mm. per minute (Gallein and Roux, 1948).

#### SPERM CONCENTRATION

The sperm concentration in buffalo bull semen is reported to vary between 631 and 1034 million per ml. (Shukla and Bhattacharya, 1949; Kushwaha *et al*, 1955). A wide range of sperm concentration varying from 210 to 2,000 million per ml. is observed in Egyptian buffaloes (Mahmoud, 1952). He attributes this variation to high sensitivity, functional disturbances and nutritional derangement. Madatov (1956) observed an average concentration of 980 million spermatozoa per ml. in buffalo bull semen. Tomar *et al* (1966), Singh *et al* (1967), Hukeri (1969) observed average sperm concentration in Murrah semen as 1140, 1230 and  $1166 \pm 114.25$  million/ml. respectively. Hafez and Darwish (1956) did not observe alteration in the sperm concentration even after successive ejaculates made after 21, 12, 8 and 2 hours where four consecutive ejaculates were obtained in each case. Prabhu and Sharma (1954) observed no significant difference in sperm concentration amongst the first, third and fourth ejaculates, while as the second ejaculate which contained the highest average concentration of spermatozoa differed to a great extent. Roy (1958) in his comparative study observed an average sperm concentration of 1261.5 million per ml. in buffalo bull semen and 1,456.8 million per ml. in the bull semen. Patel (1959) reported that in the surti buffalo bull, sperm concentration was highest from February to April and lowest from August to October. In the north of India (IVRI) spermatozoal concentration in Murrah buffalo bull semen is significantly lower in winter i.e. from November to January than in the spring (February to April). Kumar *et al* (1955) observed significant variation in sperm concentration due to sex

est the buffalo bull semen is usually light white in appearance at the first collection and deeper white at the second. Hukeri (1969) observed that majority of buffalo bull semen samples in his studies i.e. 85% were of milky white in colour and 15% creamy.

Mahmoud (1952) estimated the average viscosity of the buffalo bull semen as 451 centipoise, by using Ostwald's viscometer. The viscosity varied according to the general health of the male. He observed that semen from bulls in good condition was generally of a thick creamy consistency. The semen was thin and watery when frequent collections were made. During hot weather, the viscosity of the buffalo bull semen decreases. Observations of Sane *et al* (1955) are similar on their studies on Surti buffalo bull semen, that the colour was light milky white with a tinge of blue. Consistency was medium with marked decrease in the viscosity from July to September.

#### VOLUME

The average volume per ejaculate from a Murrah buffalo is about 3 ml. Bhattacharya and Prabhu (1955) analysing the data on the volume from 4 buffalo bulls and 19 cow bulls observed, that the average volume of semen per ejaculate was  $1.79 \pm 0.38$  and  $3.93 \pm 0.20$  ml respectively. In Indian buffaloes the volume of semen per ejaculate usually does not exceed 5 ml. The maximum quantity collected from a Murrah buffalo bull at IVRI was 8.2 ml. Mahmoud (1952) reported an average volume of 3.45 ml per ejaculate with a range of 1.2 to 6 ml from the Egyptian buffalo bulls. Hafez and Darwish (1956) observed in Egyptian buffaloes that the volume of semen ranged from 2.7 to 10 ml with a mean of 3.33

ml. In the USSR Madatov (1956) reported an average volume of 3.66 ml for buffalo bulls. Great variations are observed between ejaculates of bulls, from bull to bull and season to season. Sane *et al* (1955) observed in Surti buffalo bulls that the average volume of semen was 1.5 ml with a range from 0.5 to 3.5 ml. Considerable reduction in the volume was observed during July to August when humidity was great due to monsoon. Prabhu and Bhattacharya (1951) did not observe any significant difference in the volume of buffalo bull semen when two collections were made in rapid succession but with four the volume dropped considerably from third to fourth collections. Hafez and Darwish (1956) found no significant difference in semen volume as a result of 4 successive ejaculations in buffalo bulls when the collections were obtained at 2, 8, 12 and 24 hours interval. In buffaloes ejaculate volume may not seem to be markedly affected with increased frequency for about few days (Tomar *et al*, 1963a). Hukeri (1969) recorded average standard volume of Murrah bull semen as  $2.78 \pm 0.16$  ml. Singh *et al* (1967) reported an increase in semen volume upto 7 years of age in Murrah bulls.

**Hydrogen ion concentration (pH) of semen**—The average pH of buffalo bull semen was found to be 6.7 (Kushwaha *et al*, 1955). Seasonal variations in pH were not observed by them. Sayed and Oloufa (1957b) reported a pH of 6.8 for semen of Egyptian buffalo bulls. Prabhu and Sharma (1954) reported that when a number of semen collections were made in rapid succession from a buffalo bull, the pH changed from acid to alkaline range. A pH of 6.75 for semen collected by massage technique is reported by Singh (1956). Sane *et al*

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son, whereas Tomar *et al* (1966) did not observe and seasonal effect in Murrah bulls Singh *et al* (1967) observed an increase in sperm concentration as age advances upto 6 to 7 years

#### PERCENTAGE OF LIVE AND DEAD SPERMATOZOA IN A NORMAL EJACULATE

Prabhu and Bhattacharya (1951) estimated an average of 22.61 and 17.2% dead spermatozoa in the first and second ejaculate respectively from Indian buffaloes. Hafez and Dirwush (1956) obtained an average of 80.1% with a range of 75 to 86% live spermatozoa in four consecutive ejaculates collected in rapid succession from four Egyptian buffaloes at intervals of 24, 12, 8 and 2 hours. There is no appreciable difference in the percentage of live spermatozoa in successive ejaculates. Oloufi *et al* (1959) and Tomar *et al* (1966) found no seasonal influence on percentage of live spermatozoa in buffaloes.

**Staining method**—Several methods have been suggested to differentiate the live and dead spermatozoa. Lasley *et al* (1912) suggested the use of opal blue. Rao (1956) observed that a number of stains could be used for differential staining. The use of 5% solution of China blue, Acid green, Congo rubin, Cotton blue and Indulin gave good results. He further observed that 2% solution of Congo red or a 3% solution of a bluceloyon or a 7% solution of methyl blue or a 10% solution of light green or saturated solution of thionine were equally satisfactory for differential staining. Congo rubin and Congo red stains are to be used alone. Rest of the stains except bluceloyon and thionine are to be used along with 1% Eosin B solution. bluceloyon and thionine stains should be used with 0.8% and 0.6% Eosin solution, respectively. Reagents

like M/8 Phosphate buffer or 3% Sodium citrate (dihydrate) solution or 0.9% Sodium chloride solution may be used as satisfactory solvents for the dyes.

Hucker (1969) used staining techniques of Hancock (1951) for live and dead sperm count. The staining solution was prepared with eosin Y (B D H) 1.67 g, nigrosin 10 g and distilled water 100 ml by boiling in hot water bath in a flask with reflex condensor and then filtered. Thereafter the solution was stored in the refrigerator. Staining was done by taking one part of semen and two parts of stain in a narrow test tube. The test tube was then kept in water bath at 30°C for 2 minutes. A drop from this stained sample was taken on a clean glass slide and a uniform smear was prepared. The smears were then dried at 40°C and mounted in D P X (B D H) under coverslip. 100 spermatozoa from each of the two slides should be counted so as to have an average estimate in a given sample (Salisbury and Mercier 1945). The dead spermatozoa take up the eosin stain and appear pink while as the live ones remain unchanged. Nigrosin acts as a back ground stain in this method.

#### MORPHOLOGY OF SPERMATOZOA

The buffalo bull spermatozoa are more rectangular as compared to cow bull spermatozoa. The head of the buffalo sperm though resembles that of ram sperm is shorter and narrower (Mac Gregor 1941, Rao 1958). It is narrowest at the base and widest at a point slightly behind the anterior end. Bhattacharya (1962) cited Guha Mukherjee and Labhsetwar who measured the average length of the head, middle piece and tail of spermatozoa of Indian buffalo and cow bulls as 80, 12.5 and 51.6  $\mu$  and 100, 13.9 and 60.3  $\mu$  respectively. Lind

Table 52  
SPERMATOZOAL MEASUREMENTS (MAHMOUD, 1952)

Parts of the sperm	Mean ( $\mu$ )	Buffalo S.D.	C.V. %	Mean ( $\mu$ )	Bull S.D.	C.V.%
1 Head length	7.436	0.442	5.9	9.126	1.326	14.5
2 Head breadth (ant.)	4.261	0.520	12.3	4.732	0.494	10.4
3 Head breadth (post.)	3.172	0.442	14.0	2.730	0.520	19.4
4 Neck	0.442	0.208	47.1	0.650	0.338	51.5
5 Length of middle piece	11.648	0.936	7.9	12.558	0.624	4.9
6 Breadth of middle piece	1.092	0.286	26.2	1.066	0.286	26.8
7 Length of tail	42.882	3.042	7.1	46.28	6.084	13.2
Ratio of head breadth (ant.): head breadth (post.)	1.34	0.33	24.8	1.79	0.32	1.7

ings on the morphology of spermatozoa of Egyptian buffalo bulls and that of the native bulls studied by Mahmoud (1952) are given above (Table 52).

#### ABNORMAL SPERMATOZOA

The average percentage of abnormal spermatozoa in the Indian buffalo bull semen as reported by various authors varies between 3.0% to 17.2% (Prabhu and Bhattacharya, 1951; Prabhu and Sharma, 1954; Kushwaha *et al*, 1955; Roy, 1958). In the Egyptian buffalo bull semen, it was reported as 21%, varying between 15-32% (Hafez and Darwish, 1956). They observed that when four collections were made in rapid succession, there was an apparent increase in the percentage of abnormal spermatozoa. The most common abnormality was coiled tails, bent tails, protoplasmic droplets and tailless were other abnormalities. Double tailed spermatozoa were rare. When the interval between collections was short, spermatozoa with an enlarged midpiece, with or without tail and detach-

ed galea capitis were observed. Prabhu and Sharma (1954) observed that when the total abnormality varied from 3.0 to 11.1%, 0.3 to 1.0% were of the head, 0.46 to 1.12% were of the middle piece and 1.91 to 9.07% were of the tail. Patel (1959) reported that in ejaculates from Surti buffalo bulls at Anand, the percentage of abnormality was lowest during spring (February, March, April) and highest in autumn (August, September, October). In the Murrah buffalo bulls at Izatnagar the number of abnormal spermatozoa was lowest in the spring. The tailless spermatozoa were more during summer and autumn and a number of bent tails were observed during winter (Kushwaha *et al*, 1955). Roy (1958) observed in Murrah buffalo bulls maintained at Mathura that abnormalities were lowest during summer, rainy season and autumn. Shukla and Bhattacharya (1949) in 24 ejaculates from a single Murrah buffalo bull observed that the percentage of abnormal spermatozoa averaged  $8.3 \pm 0.26$ . Sayed and Oloufa

Table 53

DIFFERENCES IN THE BIOCHEMICAL PROPERTIES OF BUFFALO AND COW BULL SEMEN  
 Data of Roy (1959) combined with data of Luktuke and Bhattacharya (1957), Pal (1957), Sayed and Oloufa (1957, b), Pal  
 et al (1957), Ehlers, et al (1953) and Mann (1954)

Attributes	Buffalo		Bull		Statistical signi- ficance of differences
	Mean	S.D.	Mean	S.D.	
Total reducing substance (mg/100 ml)	700.40 ± 52.1	375 1480 (1)	796.59 ± 42.08		
Initial fructose (mg/100 ml)	857 (1) 355.10 ± 17.3 (i) 718 (3) (ii) 661 (3) (iii) 782 (1)		610 88 ± 38.85 (i) 775 (3) (ii) 826 (3) 952 82 ± 169 (2) 552 (2) (i) 602 (7) (ii) 413 (7) 1.99 ± 0.15		**
Fructolysis index 1st hour	(i) 478 (7) (ii) 325 (7) 1.44 ± 0.11				
Total nitrogen (mg/100 ml)	485 (1)	381-625 (1)	756 (4)		
Non-protein nitrogen (mg/100 ml)	109 (1)	85-140 (1)	48 (4)		*
Chloride (mg/100 ml)	373.20 ± 55.30 (i) 369 (7) (ii) 339 (7)		247.70 ± 26.00 (i) 328 (7) (ii) 386 (7)		
Calcium (mg/100 ml)	40.50 ± 2.10 42 (1)	35-62 (1)	25.0 ± 3.20 34 (4)	24-25 (4)	**
Ascorbic acid (mg/100 ml)	(i) 4.13 (3) (ii) 4.12 (3)		(i) 14.29 (3) (ii) 14.31 (3)		
Citric acid (mg/100 ml)	489 (1)	322-820 (1)	720 (4)	340-1150 (4)	**
Total phosphorus (mg/100 ml)	(a) 103.20 ± 8.90 (b) 95.20 ± 7.20		(a) 47.30 ± 2.50 (b) 41.80 ± 4.80		**
Organic phosphorus (mg/100 ml)	(b) 68 (1)	50.99 (1)	73 (5)		*

Attributes	Buffalo		Bull		Statistical significance of differences
	Mean	S.D.	Mean	S.D.	
Inorganic phosphorus (mg/100 ml)	(a) 6.40 ± 0.60 (b) 6.30 ± 0.40 (b) 17 (1)	14.25 (1)	(a) 5.90 ± 0.50 (b) 5.60 ± 0.40 9 (4)		
Acid soluble phosphorus (mg/100 ml)	(a) 72.20 ± 3.90 (b) 64.50 ± 2.20 (i) 60 (7) (ii) 52 (7)		(a) 29.40 ± 3.20 (b) 27.50 ± 2.90 (i) 74 (7) (ii) 51 (7)		
Total phosphates (mg/100 ml)	(a) 308.20 ± 43.80 (b) 306.80 ± 41.10		(a) 145.10 ± 11.40 (b) 167.00 ± 10.60		**
Acid phosphatase activity (Bodansky unit)	(a) 251.70 ± 37.20 (b) 266.50 ± 42.20		(a) 133.80 ± 14.10 (b) 151.60 ± 18.50		*
Alkaline phosphatase activity (Bodansky unit)	66.80 ± 10.50		122.00 ± 14.80		**
Oxygen uptake ( $\mu$ l O <sub>2</sub> )/5 × 10 <sup>8</sup> sperm diluted with saline (1:1)	48.30 ± 6.0		80.70 ± 2.40		**
Oxygen uptake ( $\mu$ l O <sub>2</sub> )/5 × 10 <sup>8</sup> sperm diluted phosphate buffer (1:1)	65.10 ± 10.60		112.80 ± 11.90		**
Oxygen uptake ( $\mu$ l O <sub>2</sub> )/5 × 10 <sup>8</sup> sperm diluted with Krebs buffer (1:1)					

(1) Data of Pal (1957)

(2) Data of Luktuke and Bhattacharya (1957)

(3) Data of Pal et al (1956)

(4) Data of Mann (1954)

(5) Calculated from Mann's data

(6) Data of Ehlers et al (1953) cited from Mann

(7) Data of Sayed &amp; Oloufa (1957b)

(i) First ejaculate

(ii) Second ejaculate

(a) In whole semen

(b) In seminal plasma

(i) In adults

(ii) Young

\* Significant at 5% level

\*\* Significant at 1% level



(1957a) observed an increase with abnormal percentage of spermatozoa as the frequency of collection is increased from once a week to 3 times a week. Singh *et al* (1967) found decrease in the proportion of abnormal sperms as the age of Murrah bulls advances. The abnormal spermatozoa of buffalo bulls are classified similarly to that of cow bulls.

### Biochemical characteristics of buffalo bull semen

Comparative data on the biochemical characters of buffalo bull and cow bull semen are given in Table 53.

Roy (1959) observed that the fructolysis index both in the buffalo and cow bull showed a highly significant correlation with half life of spermatozoa. Similarly a highly significant correlation between fructolysis index and full life of spermatozoa was also observed for the bull semen. Initial fructose content showed a significant correlation with full life of spermatozoa in both the species. Seasonal variation in fructolysis of buffalo semen was observed by Sen Gupta *et al* (1963) as higher during spring season (2.19) and lowest during summer season (1.26).

### Bacterial contamination of semen

Mahmoud (1953) studied the microflora present in the prepuce and semen of buffalo bulls in Egypt. Gram negative cocci and gram positive organisms were detected from cultures made from the preputial washings, fresh semen or stored diluted semen. Diptheroids, bacillus fusiformis and smegma bacilli were the most commonly observed gram positive organisms. By the addition of sulphanilamide, most of the gram positive organisms disappeared and gram negative bacteria diminished consider-

ably. However, more effective control on bacterial contamination was observed with the addition of penicillin. The seminal microflora content can be kept at a very low level provided strict hygienic precautions are practiced.

Kodagali (1973) investigated the degree of bacterial contamination in the semen of 66 Surti buffalo bulls and his observations were as follows—Coryne bacteria species 33.3%; Staphylococcus coagulase negative 13.0%; Anthracoids 10%; Pseudomonas aeruginosa-10%; E. coli-6.6%; E. freundi-6.6%; Streptococci species non-haemolytic-3.5%; Sarcina species 3.5%; Yeast unidentified-3.5%; Unidentified gram negative rods-10.0%.

### Seasonal variation in semen quality

It is observed that the quality of semen of Murrah and Surti buffalo bulls in India is good during spring and fairly good during winter. There is a relative decline in the quality of buffalo semen during summer and autumn, similar to that of bulls, rams and goats (Malkani, 1954; Kushwaha *et al*, 1955; Roy, 1958). The buffalo bull is very sensitive to extreme cold and heat and cannot adopt itself to colder climate like cattle and therefore the possible decline in semen quality due to seasonal variations is not surprising (Kaleff, 1912). Buffaloes kept in temperate zones are not subjected to seasonal variations in semen quality (Malkani, 1954).

During summer, buffalo spermatozoa have a very high  $O_2$  uptake immediately after ejaculation and there is a sharp drop within 15 to 30 minutes followed by a continuous rapid decline. In contrast to this  $O_2$  utilization of spermatozoa during winter is sustained at a very high level for about 2 hours. It is also observ-

Table 54

## CHARACTERS OF BUFFALO AND COW BULL SEMEN DURING DIFFERENT SEASONS\*

Figures in bracket indicate observations on *Bos taurus* maintained in India

	Winter	Spring	Summer	Rain	Autumn
1. Sperm density per ml $\times 10^6$	1295.7 $\pm$ 121.7 (1638.8 $\pm$ 65.2)	1563.2 $\pm$ 191.6 (1537.7 $\pm$ 100.7)	1277.4 $\pm$ 117.6 (1456.2 $\pm$ 106.4)	1040.3 $\pm$ 114.7 (1277.7 $\pm$ 119.7)	1098.2 $\pm$ 125.3 (1037.5 $\pm$ 189.9)
2. Living sperm per ml.	896.8 $\pm$ 100.3 (1190.3 $\pm$ 66.2)	1031.9 $\pm$ 172.9 (1139.0 $\pm$ 55.0)	798.4 $\pm$ 89.7 (1030.4 $\pm$ 119.4)	809.3 $\pm$ 81.6 (1070.8 $\pm$ 108.1)	952.8 $\pm$ 125.7 (825.1 $\pm$ 162.4)
3. Percentage of living sperm	69.0 $\pm$ 3.0 (73.9 $\pm$ 2.7)	62.2 $\pm$ 3.9 (75.1 $\pm$ 2.8)	63.2 $\pm$ 4.6 (67.0 $\pm$ 4.6)	79.2 $\pm$ 2.3 (77.5 $\pm$ 4.7)	82.8 $\pm$ 1.8 (72.4 $\pm$ 3.3)
4. Percentage of total abnormal sperm	23.6 $\pm$ 2.1 (22.3 $\pm$ 2.3)	21.1 $\pm$ 2.5 (14.0 $\pm$ 1.9)	15.2 $\pm$ 2.2 (11.6 $\pm$ 2.5)	10.1 $\pm$ 1.1 (12.7 $\pm$ 2.8)	12.4 $\pm$ 2.9 (17.8 $\pm$ 3.8)
5. Percentage of living abnormal sperm	20.6 $\pm$ 7.4 (12.6 $\pm$ 4.6)	13.2 $\pm$ 2.0 (8.9 $\pm$ 1.4)	5.0 $\pm$ 0.3 (4.3 $\pm$ 0.9)	6.0 $\pm$ 0.8 (5.9 $\pm$ 1.3)	3.9 $\pm$ 0.7 (9.4 $\pm$ 4.3)
6. Fecundity index last hour	1.81 $\pm$ 0.16 (2.09 $\pm$ 0.19)	1.17 $\pm$ 0.17 (1.86 $\pm$ 0.24)	1.31 $\pm$ 0.17 (1.54 $\pm$ 0.18)	1.73 $\pm$ 0.37 (1.66 $\pm$ 0.22)	1.27 $\pm$ 0.24 (1.81 $\pm$ 1.17)

\*Roy (1935) has classified the seasons in India not according to months of the year but on climatological considerations; broadly speaking the seasons in relation to months of the year are as follows:

1. Winter — from November 20 to February 10
2. Spring — from February 10 to April 15
3. Summer — from April 15 to July 15 some time to the end of July
4. Rain — from July 15 or end of July to October 7
5. Autumn — from October 7 to November 20

ed that during respirometric activity there is a heavy mortality in the spermatozoa population and the maximum mortality occurs during first 30 minutes (Roy, 1959; Bhattacharya, 1962). Increased or decreased activity of the thyroid and testes coincide with the periods of production of semen of better or poorer quality (Bhatnagar *et al*, 1955). Seasonal variation in seminal attributes have been mentioned in Table 54.

In general it is observed that the thermoregulatory mechanism in the buffalo bull is comparatively less efficient than that in the cow bull. This may be the possible reason for seasonal variations in the quality of semen.

#### **Dilution and storage of buffalo bull semen**

Srivastava and Prabhu (1956) experimented with six diluters for extending buffalo bull semen i.e. egg yolk phosphate, egg yolk citrate, autoclaved milk, Kampschmidt's glucose sodium bicarbonate egg yolk diluter with addition of sulphamezathine, active principle of egg yolk in phosphate buffer and spermasol. Encouraging results were noticed with glucose sodium bicarbonate diluter, followed by spermasol for storage extending over a period of a week. Gokhale (1958) reported that addition of sulphamezathine in Kampschmidt's diluter has extended the period of motility of buffalo bull semen to 127.2 hours appreciably longer than in egg yolk citrate (100.2 hours) and egg yolk phosphate (77.5 hours) and better conception rates (52.29 per cent) as compared with egg yolk citrate (40 per cent) and egg yolk phosphate (41%). Roy *et al* (1955) and Chetty (1963) observed a higher motility rating with glycine added to egg yolk citrate diluent and that the

conception rate was also slightly better than that of egg yolk citrate. Singh and Tomar (1959) evolved a sodium bicarbonate glucose fructose buffer with egg yolk for storage of buffalo semen and reported that this buffer maintained motility score of 3 for a longer period, than in egg yolk citrate or egg yolk glycine diluter. Veeramani Ayyar (1952) tried boiled milk with citrate buffer as a diluter both for cow bull and buffalo bull semen and observed that it serves equally good medium for storage as in egg yolk diluter. Desai and Saha (1959) reported that the buffalo bull semen samples with 2.5 to 3.0% sodium citrate and 50% egg yolk not only maintained the motility at higher level but also prolonged the life span of sperms by 24 to 48 hours respectively, on storage. Iyer (1964) found that egg yolk citrate diluter containing M/15 molar strength of sodium citrate was more suitable for buffalo bull semen than M/10 or M/20. Mahajan and Sharma (1963) found egg yolk glucose bicarbonate diluter significantly superior to yolk phosphate, yolk citrate, yolk boiled milk and yolk glycine diluters in dilution of semen 1:10 and 1:50 and preserved a desired motility for artificial insemination for at least 3-4 days. Joshi (1961) observed better results with milk yolk diluent for extending buffalo bull semen than either with whole milk or glucose sodium bicarbonate diluter upto a period of 72 hours at 5°C. Tomar and Desai (1961-C) made a comparative study of various diluters at 5 to 7°C and observed that in egg yolk glycine heated skim milk and egg yolk fructose heated skim milk, the motility of the spermatozoa was +3 for an average period of 8.1 days and 7.65 days respectively. Kale (1963) found glucose whole milk yolk and glucose whole milk carbonate as superior

diluters compared to whole milk egg yolk, whole milk and whole milk citrate diluters. Hukeri and Kaikini (1973) observed that egg yolk skim milk glycerol and egg yolk skim milk diluters were superior to egg yolk glucose fructose sodium bicarbonate, egg yolk citrate and egg yolk glycine diluters for preservation of Murrah buffalo bull semen over a period of 120 hours at 5°C. Bhosrekar and Ganguli (1974) reported that the buffalo spermatozoa maintained 54% motility in the heated Citric Acid Whey (CAW) at 30°C with the dilution ratio of 1:10.

Chaube and Sengupta (1974) observed the conception rates in citric glycine diluter and tris + citric acid + glucose + cysteine diluter as 65% and 59.7% respectively. In both these diluters the storage interval was, 0 to 32 hours (at 1 to 2°C).

#### DILUTION RATE

The rate of dilution of buffalo bull semen at most of the A.I. Centres in India usually does not exceed 1:10 and occasionally ranging from 1:5 to 1:30. Better fertility rates are claimed when fresh diluted semen samples are used (Sane *et al*, 1955). In general, it is observed that the diluted buffalo bull semen stored at 5°C maintains +3 motility on an average for a period of 3 to 4 days.

#### Use of Antibiotics

Use of antibiotics for the control of bacterial growth is highly recommended in preserving buffalo bull semen in the proportion of 1,000 I.U. of crystalline penicillin G Sodium and 1000 µg of Dihydrostreptomycin per ml. of the diluter.

#### Insemination Technique

Technique of insemination in the buffalo is similar to that in the cow. For the beginners, use of vaginal speculum is advised to locate the cervix and to judge the state of oestrus. Recto-vaginal technique is the most convenient method. Buffaloes are more sensitive for manipulation per rectum and easily bleed on a little hard pressure exerted for palpating the genitalia in general and ovaries in particular. All possible care should therefore be taken to prevent straining.

#### Sperm travel in the genital tract of the buffalo

Rao (1954) observed that the buffalo bull spermatozoa required 3 minutes 20 seconds on an average following insemination to reach the anterior third of the fallopian tubes and the sperm survive in the reproductive tract for 36 to 48 hours. This is similar to that in the cow.

Difficulties are experienced in detecting heats in buffaloes owing to pronounced variations in the symptoms of heats expressed. Difficulty is also experienced in judging the exact time when insemination should be done. Very deceptive signs of heats are noticed in the so-called silent heat pattern and it is difficult to say if the heats would be ovulatory or the follicle will become atretic resulting in anovulatory heats. At times it is difficult to pass the insemination pipette through the vulval opening to the anterior portion of the vagina especially in heifers. Pronounced seasonal variation in the oestrous phenomenon is a great handicap which has a bearing on the low conception rate commonly encountered in buffaloes under A.I. method of breeding. It has also been observed that the buffaloes are

more sensitive to touch and vaginal contractility is comparatively greater than in the cow. Straining in buffaloes is markedly observed during rectal palpation and vaginal manipulation. In buffaloes perineum is not prominent, cervix is rigid and hence the difficulty in passage of the pipette. All possible care should therefore be taken not to force the inseminating pipette to avoid injuries to vaginal mucous membrane and cervix. Physiological hypertrophy of the cervix from calving to calving which is markedly observed in cows is not commonly encountered in buffaloes (Sane and Desai 1956).

Villegas (1928) reported that in Philippines high sexual activity is observed in buffaloes during the rainy season and cooler months. In Egypt Hafez (1955) and Asker and El Itriby (1958) observed that majority of the buffaloes remain in anoestrous condition during summer months (April to July) and they return to sexual activity in autumn (August) and majority of services occur from November to February. It has been observed by various workers under Indian conditions that about 80% of the total number of oestrous periods recorded in buffaloes were from October to March and only 20% during the period from April to September. The higher temperature and humidity coincide with the period of lower sexual activity (Goswami and Nair 1964).

Ovulation in the buffalo occurs from 1 to 30 hours after the end of heat, the average time being 11-10 hours. Rao (1954) observed that on an average the duration of oestrus was 36 hours and ovum in the buffalo was fertilizable 24 hours before the end of oestrus at the earliest and 10 hours following the termination of heat.

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# Chapter 41

## Organisation of Artificial Insemination Service in Cattle & Buffaloes

In almost all the countries of the world, considerable attention has been focused during the recent years in breeding cattle, buffaloes and sheep by artificial insemination due to numerous advantages which it affords in bringing about rapid genetic improvement of farm animals on a mass scale through

Experience gained so far in India and other countries of the world show that small units prove costly in every way. Large units are much more economical.

The advisability of setting up of such an organization depends on various factors, as under



with regard to the numbers of the breedable cows, heifers, male and female calves, breeding bulls, bullocks with specific mention of the breed and whether they are of the milch, draught or beef type. More-or-less similar type of census is also necessary for the buffalo class. The cattle census will give a fair idea as to whether it is advisable to establish such type of organisation in the district proper with a full fledged bull centre for collection and processing of semen. The type of breed and number of bulls to be maintained at a full fledged bull centre depends on the number of breedable cows in the area under operation. Experience shows that the minimum number of bulls of any breed should not be less than four. It is advisable that annually at least 2,000 cows should be bred to each bull. This however depends on the quality of sire. Progeny tested sires should be used with great advantage for large number of conceptions.

Considering the economics and area of operation, it is advisable that there should be atleast 100,000 breedable cows in the project area.

In the Intensive Cattle Development Projects in India, mass scale developments for cattle improvement have been implemented with an objective to enhance the milk production, by selective breeding and by extensive use of superior sires through the method of breeding by Artificial Insemination. Cross breeding of the indigenous breeds of cattle with the use of frozen semen from exotic Holstein, Jersey, Brown Swiss and Red Dane bulls is also undertaken. In addition to the breeding programme other important items such as fodder production, hay making, supply of concentrates through feed factories, marketing of milk, and sale-purchase of animals

have also been carefully planned to boost up the progress in milk production. Each project is having an approximate number of 100,000 breedable cattle and buffaloes and bull centres have been opened in suitable places for supply of semen in the project areas. By and large it can be said that the project breeding association can be considered as a very large herd composed of small units distributed in several places. It is possible through such large breedable cattle population to have the sires progeny tested.

The organisation of full fledged centres and sub-centres is comparable with the two common types existing in the United States viz. (1) Central or Federated organisation (2) Local breeding organisation. The central or federated organisation operates on a statewide or district-basis. It may be co-operative or private enterprise. Bulls of the required type are maintained by this central organisation and semen is despatched to a number of local breeding organisations. The local units are usually co-operative associations which obtain the semen of superior bulls from the Federated organisation. This proves beneficial to them in every respect.

The functions of federated or district organisations are as follows:

1. Survey of cattle population.
2. Selection of suitable site for bull centre.
3. Appointment of office bearers and technical staff.
4. Enrolment of farmers as members.
5. Purchase of bulls of the requisite type.
6. Construction of buildings for bull centres.

7. Purchase of laboratory equipments.
8. Actual execution of work in a radius of 15 kilometers and transport of semen to sub centres.

9. Follow up of insemination work, sexual health control, progeny testing of bulls and maintenance of records.

10. Fees to be charged for insemination depends on the type of organisation.

The Ministry's and Milk Marketing Board Centres in England charge £2 per insemination, in U.S.A. the fee is 5 to 8 dollars and 44 Swedish Kroners in the Scandinavian countries. In India, the State artificial insemination service is mostly free. The Bharatiya Agro-Industries Foundation (BAIF) Organisation which has started large number of A.I. Centres in India with the objective of crossbreeding of cattle, is charging Rs. 150/- per conception with frozen semen from progeny tested exotic bulls.

11. (i) The semen producing centre is supposed to produce semen from purebred dairy bulls so that the off-springs from registered dairy cattle herds are eligible for herd registry in the respective breed societies.

- (ii) Semen must be produced from bulls registered in the respective herd books of the pure bred dairy cattle.

- (iii) As far as possible at least 50% of the bulls should be proven ones.

- (iv) As far as possible farmers may be allowed to have their choice of bulls. This may however be done with levy of an extra fee. The scope for

such a choice is however limited unless otherwise large number of bulls are maintained for semen production.

- (v) It is necessary to use the bulls to their maximum capacity and either transfer them to some other centres on exchange basis or on cash, after reasonable number of inseminations to avoid dangers from inbreeding.

- (vi) Upkeep of the bull centres is very important.

12. An artificial breeding organisation may have to face a number of problems few of which are as follows:—

- (i) Difficulties commonly experienced to obtain proven bulls.

- (ii) Farmers have to be convinced that it is never possible to conceive all cows with one or two inseminations, since few problem cows would always be there in any herd.

- (iii) Necessity of subjecting such herds for investigations where condition of animals is excellent but conception rate is poor.

- (iv) Scarce cattle population in an area may increase the overall cost of inseminations.

- (v) In the event of an outbreak of Foot and Mouth Disease, the inherent danger of the inseminators transmitting the infection from place to place during their visit to farms should not be ignored.

13. In order to get farmers interested, it is necessary to supply them all possible information with regard to the

type of bulls used, their pedigree records and conception rates. By holding periodical meetings, they should be acquainted with the breeding problems and diagnosis of heats in cows for prompt report to the A.I. Centres in order to inseminate cows in good time. Importance of sexual health control to gain higher fertility has to be impressed on their minds.

11. An initial budget has to be prepared for expenditure on the cost of land, construction of buildings,

farm, bull yard and laboratory equipment; purchase of bulls, purchase of feeds and fodders, staff salaries, purchase of conveyance and transport charges.

*It takes considerable time for the implementation of a scheme and as such it is advisable to prepare the initial budget for a period of 5 years. If the service is free, the entire expenditure shall have to be borne by the central organisation. In case, fee is levied the economics has to be worked out to find if the receipts can balance the expenditure.*

## Chapter 42

### Artificial Insemination in the Equine

The method of breeding by Artificial Insemination is not much practised in horse breeding except in few countries. Successful results have been reported but its use in the regular breeding programme is not found very beneficial on account of several factors such as delicate nature of the spermatozoa, poor keeping quality and effects of agitation during transport. Lower fertility is usually observed in mares under small scale stud conditions. Moreover, prolonged oestrus period in the mare as compared to cattle and sheep also pose difficulties in the diagnosis of the appropriate time for insemination.

Horse population is decreasing from year to year due to mechanisation of farm work and transport. In a few countries the A.I. method of breeding is used as supplementary to natural mating, with the object of promoting higher pregnancy rate and less stress on Stallions. Legislation by breed societies in certain countries prevents use of this method for breeding mares. In Great Britain thorough bred foals obtained by A.I. are not eligible for entry into the stud book. In U.S.A. rules permit the use of this method as supplementary to natural mating, but, breeding done by A.I. is not permissible (Schell, 1948). In the breed improvement schemes Russia

has taken immense advantage of this method. Japan, Brazil, Spain and Greece are the other countries to make use of this method for horse breeding. In India, Sane *et al* (1953) used this method of breeding supplementary to natural mating chiefly with the objective of overcoming subfertility. A number of mares on stud farms in and around Poona were inseminated but the results were not very encouraging since only the mares which were repeaters or infertile were offered for inseminations.

#### **Special features of the physiology of reproduction in the Equine**

The pattern of reproduction is rather complicated in the mare and donkey mare and as such results obtained with Artificial Insemination are less favourable compared to the other livestock and poultry. Several difficulties are usually encountered in survival of equine sperm during extension and preservation.

Mares remain in heat for many days. This necessitates repeated inseminations in the same heat period. It is difficult to forecast the exact time of ovulation as it occurs independently 24 to 72 hours before cessation of heat symptoms. It is difficult to predict the

time of ovulation since the interval between onset of oestrus and ovulation is highly irregular. The viability of the spermatozoa in the intra uterine tract of mare is only for about 24 to 48 hours and of an unfertilized ovum for about 5 to 8 hours. Therefore services done very earlier or very late in oestrus period usually prove ineffective. In natural conditions a stallion usually covers a mare several times during the heat period. In a small stud consisting of not more than 20 to 30 brood mares when stallion gets an opportunity of service in good time high conception rates from 80 to 90 percent can be obtained. Whereas with the usual method of hand breeding when mare is shown to the stallion or Jack Ass once only it results in a much lower conception rate. This is on account of the fact that it is often difficult to approximate service with ovulation. Considering the complexity of heat periods and necessity of number of services the method of breeding by Artificial Insemination can be considered as ideal for maximal utilisation of superior sires on large number of mares with minimum exhaustion and high percentage of conception rates. In breeding of mares the special point of interest is that the mares to be bred should be available for service during the entire period of their heat without which frequent inseminations would be impossible.

### Physiology of Semen

Unlike with bovine or ovine spermatozoa the poor viability of equine spermatozoa is partly due to poor initial sugar content of semen and its early exhaustion during metabolism. Stallion semen remains highly alkaline in nature

due to high proportion of accessory fluids is a result of which natural anaerobiosis due to acidification by lactic acid formation does not occur. The accessory fluids also have a high concentration of salts which stimulates motility of the sperm cells thus hastening their death by exhaustion. The higher concentration of salts also exerts adverse effect on the capsule of the sperm head and causes its destruction. As compared to bull and ram sperm the capsule of stallion sperm is weaker.

In the mare the semen is deposited in the uterus either in natural service or by artificial insemination and not in the sperm life preserving area of the cervix. This is on account of the fact that cervix in the mare is not anatomically suitable to serve as a reservoir for holding the semen. The uterine environment is usually not favourable for prolongation of the life of the sperm. Semen has therefore to be treated with proper diluents for prolongation of its life in vitro and in the uterus after insemination.

It is experienced that sperm survival in stallion and jack semen is better when the semen is diluted in a ratio of 1:3 to 5 parts of dilutor. Survival time decreases with increase in dilution rate. With dilution rate of 1:10 survival period is found to be poor.

Fairly good conception rates have been claimed by using not less than 1 to 2 billion sperm per insemination. In mares which have undergone number of pregnancies and which are particularly heavy in size large volumes of diluted semen 10 to 20 ml are necessary for insemination for further transport of the sperm to the site of fertilization in good time. This volume may have to

be increased to 30 to 40 ml. Due to unsatisfactory methods of preservation of stallion semen it is advisable to use only freshly diluted semen.

### Collection of Semen

For collection of semen from a stallion and jack various methods are employed such as collecting the last portion of the ejaculate when the stallion dismounts, scooping semen from the vagina of the mare after the service and similar other methods. These methods are not satisfactory as the semen gets contaminated with vaginal secretions and bacteria which makes it unsuitable for preservation and storage. In case, mare harbours any infection, such a collection becomes dangerous.

### Artificial Vagina

Artificial vagina similar to that used for bulls is considered ideal for collection of semen from stallions and jacks. The Cambridge or Russian model and the Missouri or American model are considered suitable.

### Russian Model

For the medium size stallion, the length of the artificial vagina is 64 cm. with a diameter of 13 cm. at the opening which is reduced at the distal end to 8.5 cm. This is further reduced by means of a shoulder (core) 4.5 cm. in length which serves as a support for the collecting bottle of 7.5 cm. in length and of 200 ml. capacity. An adjustable valve is fitted which enables the air pressure in the water space. The ratio of air to water is considered to be important. The outer container is made up of aluminium and has a standard cambridge liner (Skatkin, 1957).

### American Model

The American or Missouri model described by Lambert and McKenzie (1940) is more complicated than the Russian model. It consists of an outer cylinder of 15 cm. in diameter and 45 cm. in length. It is made up of thick pliable rubber. The inner collecting tube is vulcanised to the outer tube at both the ends. A sphincter like rubber band is fitted near the mouth of the liner at the proximal end to simulate pressure of the labial sphincters. At the distal end after the connection to the outer tube, a continuation of the inner tube tapers down so as to allow the fitting to the collecting glass bottle. In order to obtain correct pressure, air and warm water are pumped into the cavity between inner and outer tubes. Several modifications have been made in order to obtain an equilibrium of pressure between the walls of the vagina and the pumping apparatus to effect better feel in order that the donor gets sensation similar to that of natural service (Fig. 152a).

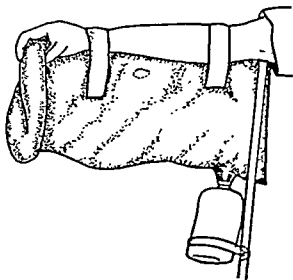


Fig. 152(a). Artificial Vagina for Stallion (Mississippi Model).

Beiliner (1910 and 1945) suggested certain modifications based on the fact that the main stimulation of the ejaculatory nerve depends upon the pressure of the vaginal walls on the glans penis and not on the vulval pressure. The pressure in the artificial vagina is adjusted according to the size of the sire. Individual preference should be considered. The temperature of the artificial vagina is regulated between 41-45°C. The inner side of the artificial vagina is lubricated with non-spermicidal lubricant. At the time of collection a mare in heat is controlled with proper restraint with hobbles. The tail of the mare should be wrapped by a clean bandage. Stallions and Jacks can also be trained for collection on a dummy.

### Semen Collection

#### REACTION TIME

Wierzbowski (1958) observed that the reaction time from copulation to ejaculatory reflex was about 13 seconds under natural service and 16 seconds with artificial vagina. More mounts are necessary when artificial vagina is used for collection as compared to natural service. Parsutin and Rumjanceva (1953) recommended that it is better to allow the stallion to mount several times before actual collection in order to obtain optimum quality and quantity of semen. This gives effective stimulation for the production of a fairly good ejaculate. They also observed better results with a mare than when a dummy was used. Stallions vary with regards to their reaction to the artificial vagina. In case the temperature of the artificial vagina is below 37°C, stallions would refuse to serve. Similarly too high or too low pressure in the vagina will also distract attention of the stallion.

The artificial vagina is held firmly against the flank of the mare and it should not be pushed towards the stallion so that necessarily the stallion has to make forward thrust as during natural service to gain ejaculation as soon as the glans penis comes in contact with its anterior wall. The penis is then guided by hand and directed into the artificial vagina. In the stallion and Jack about 10 seconds are required for ejaculation to occur and the seminal fluid is expelled in several fractions. This is in contrast to that in the bull, where ejaculation is obtained with one sudden thrust.

### Volume, colour and consistency

The first fraction of the ejaculate is of watery consistency and is devoid of spermatozoa. In the subsequent main phase to follow, the ejaculate comes from the testes and possess high concentration of spermatozoa. This main fraction appears milky white due to the presence of spermatozoa. During the third and the final phase a glairy, viscous material resembling white of an egg is expelled. This is the secretion from seminal vesicles and the Cowpers glands which contribute to the bulk of the seminal fluid and this contains no spermatozoa.

The secretions from the accessory glands approximately make up half of the volume of the total ejaculate in draft stallion but in the semen of saddle stallion and jack it is present in smaller amount. The total average volume per ejaculate is 70(60-100 ml.). The volume can vary from 30 to 300 ml. per ejaculate. MacLeod and McGee (1950) observed in 23 thorough bred stallions that the volume of semen was  $60 \pm 20$  ml. The ejaculate of stallion is whitish,

opaque and often of a gelatinous consistency.

### Sperm concentration

The average sperm concentration is 100-150 millions/ml. MacLeod and McGee (1950) observed sperm concentration of  $234 \pm 108.0$  millions/ml. in the semen of stallions.

### pH of the Semen

The average pH is 7.33. Becnikov (1955) reported pH of 6.97 to 7.71 on his studies of semen from 29 stallions. He observed that the pH was unaffected by age and it showed no correlation with motility, viability, volume or concentration or number of spermatozoa per ejaculate. MacLeod and McGee (1950) recorded pH between 7.2 and 7.8 with an average of 7.4 in their work on semen studies of thorough bred horses.

### Sperm Morphology

Measurements of the stallion spermatozoa recorded by Nishikawa and Waide (1951) and Nishikawa *et al* (1951) are as follows:

Total length of spermatozoa	60.55 $\mu$	(57.4—63.0)
Length of head	7.0 $\mu$	(6.1—8.0)
Width of head	3.9 $\mu$	(3.3—4.6)
Length of		

Wagenaar and Grootenhuis (1953) in their observations on 35 stallions and 3,000 mares reported that for obtaining good conception rates stallion must produce 10,000 spermatozoa per mm. with 65% of normal morphology.

### Biochemical composition of semen

The testes of the stallion are characteristic for producing large quantities of oestrogen. On certain occasions the oestrogen content in the stallion urine occasionally exceeds the amount present in the urine of pregnant mares (Zondek, 1931). Ejaculation from the stallion as with the boars is delivered in fraction which differ in origin and composition. Ejaculation begins with the delivery of the pre-sperm fraction which is thin and watery. It does not contain spermatozoa, ergothioneine or citric acid. In a few seconds sperm rich second fraction follows, which is milky in appearance and not gelatinous. In addition to the high sperm concentration it also contains high concentration of ergothioneine and a small quantity of citric acid.



Table 55

## NON-ENZYMIC COMPONENTS OF STALLION SEMEN (As per Mann, 1964)

Results (1) and (2) were reported by Slovtzov (1916) and Milovanov (1938) respectively on whole semen, results (3) by Yamane (1920) on sperm and seminal plasma separated by centrifugation, only 0.7—3.5% of the ejaculates was represented by the spermatozoa and the rest was seminal plasma, the volume of ejaculates varied from 40 to 130 ml., sperm density from 92.6 to  $283.4 \times 10^3/\text{ml.}$  or from 3.7 to  $22.7 \times 10^6/\text{ejaculates.}$  Data (4) by Bernstein (1933), (5) by Ilyasov (1933) and (6) by Shergin (1933, 1935).

	Whole semen	Sperm	Seminal plasma
Specific gravity	1.0117—1.0149(3)	1.0975(3)	1.0116(3)
Freezing point (0°C)	—0.5570	—	—0.615(3)
Dry material (g./100ml.)	4.295(1)	20.255(3)	2.541(3)
Ash (g./100ml.)	0.915(1)	1.760(3)	0.914(3)
Bicarbonate (ml. CO <sub>2</sub> /100ml.)	24(6)	—	—
Calcium (mg./100ml.)	20(2)	122(3)	26(3)
Chloride (mg./100ml.)	476(2)	11(3)	448(3)
	86—443(4)	—	—
Cholesterol (mg./100ml.)	4.2(1)	—	—
Creatine (mg./100ml.)	3.0(5)	—	—
Creatinine (mg./100ml.)	12.1(5)	—	—
Lipid (mg./100ml.)	172(1)	—	—
Magnesium (mg./100ml.)	3.0(2)	43(3)	9(3)
Non-protein nitrogen (mg./100ml.)	55.0(2)	—	—
Phosphorus (mg./100ml.)	19.0(2)	495(3)	16(3)
Potassium (mg./100ml.)	62.0(2)	—	103(3)
Protein (g./100ml.)	1.043(2)	—	—
Sodium (mg./100ml.)	68(2)	—	275(3)
Sulphur (mg./100ml.)	3(2)	32(3)	8(3)

The chemical characteristics exhibited by the semen of Jack-Ass are similar to those of the stallion semen. It has high content of lactic acid contributed by seminal vesicle than the other accessory organs.

Mann (1954) observed that unlike in the semen of many other species, the concentration of fructose in semen of stallion is very low. Its average concentration is 50 mg./100 ml. and 224 mg./ml. in the semen of stallion and bull

Table 56  
STALLION SEMEN

As per Mann (1961) further data on the composition of semen based on analysis of ejaculates collected from the same Shetland Pony on different occasions. (Data by Dawson *et al* (1957). Hartres (1957), Mann (1959) and Mann *et al* (1956). Figures in round brackets refer to the number of ejaculates analysed, figures in square brackets give the range of variations).

	56	[27—100]
Volume of ejaculates (12) (ml.)	30.7	[22.7—37.5]
Dry weight (9) (mg./per ml.)	19.6	[13.2—26.2]
Ethanol soluble material (4) (mg./ml.)	113	[40—172]
Sperm density (11) (million cells/ml.)	26.1	[8.1—53.0]
Citric acid (15) (mg./100ml.)	7.6	[3.5—13.7]
Ergothioneine (15) (mg./100ml.)	17.3	[12.0—27.8]
Phosphorus, total (3) (mg./100ml.)	11.2	[11.5—22.1]
Phosphorus acid soluble (3) (mg./100ml.)	32.7	[16.9—42.1]
Carbohydrate, ethanol soluble and anthrone reactive (5) (mg./100ml.)	8.1	[1.9—16.2]
Carbohydrate, ketose-reactive (8) (mg./100ml.) (in terms of fructose)	2.1	[0.3—6.3]
Carbohydrate, fructose i.e. ketose-reactive and yeast fermentable (8) (mg./100ml.)	12.1	[9.2—15.3]
Lactic acid (3) (mg./100ml.)	1.0	—
Urea (1) (mg./100ml.)	1.3	[0.3—2.4]
Ammonia (4) (mg./100ml.)	3.4	—
Glycerylphosphorylcholine (2) (mg./100ml.)	113	—
	31.2	[19.0—47.3]
Inositol (6) (mg./100ml.)		

Table 57  
JACKASS SEMEN

Composition of whole ejaculated semen, epididymal semen, ampullar semen, seminal vesicle secretion and prostatic secretion all obtained from the same animal (Mann, Minotakis and Polge, 1963). Results on whole semen, based on the analysis of several ejaculates, are averages, range is given in brackets, number of analysed ejaculates is given in square brackets, the contents of the epididymides, ampullae, seminal vesicles and prostate were obtained at slaughter four days after the last ejaculate had been collected.

Whole semen				
Volume (ml.)		69.1	(36—120)	[14]
Sperm concentration (million/ml.)		229	(100—475)	[11]
Fructose (mg./100ml.)		2.8	(1.9—3.8)	[2]
Lactic acid (mg./100ml.)		44.4	(22.3—77.4)	[5]
Ergothioneine (mg./100ml.)		3.4	(1.9—5.0)	[4]
Citric Acid (mg./100ml.)		7.4	(3.6—10.4)	[4]
Acid soluble phosphate (mg.P/100ml.)		19.7	(17.9—21.6)	[4]
Chloride (mg. NaCl/100ml.)		757	(748—773)	[4]
Organ contents (mg./100ml.)				
	Epididymal semen	Ampullar semen	Vesicular secretion	Prostatic secretion
Lactic acid	54.8	52.8	319.0	81.2
Ergothioneine	0.0	50.4	2.0	0.0
Citric Acid	0.0	0.0	72.0	0.0
Acid soluble phosphorus	260.0	32.7	22.0	12.0

#### Dilution, preservation and storage of semen

It is quite common to use undiluted stallion semen for insemination of mares when the number of mares to a stallion is very limited. When large number of mares are assigned to one stallion, the semen has to be diluted and extended for use either for a single or repeated insemination.

The equine spermatozoa are very delicate as compared to those of other domesticated animals. This has been attributed to the relatively weak lipid capsule of the sperm cell. Rubber and

aconite are highly lethal while as polythene is practically harmless to equine spermatozoa on contact.

On account of the sensitivity of the stallion sperm it is recommended that dilutor should be added to semen as soon as collection is obtained. This should be done regardless of whether it is required for immediate use or for storage and transport. The dilutor should be warmed to body temperature and added gradually and mixed uniformly by slow rotating movements. Stirring with glass or stainless steel rod should be avoided.

The viscous fraction interferes with dilution and storing qualities of semen. It is therefore advisable to separate it from the sperm bearing fraction immediately after collection. This can be done by pushing out the glairy material with a glass rod. Diluted semen is held in water bath at 20-27°C and is used for insemination within a few hours of the extension. In case it becomes necessary to use part of the diluted semen next day, it is stored under refrigeration in single dose containers and warmed before insemination. This procedure facilitates maximal use of superior germ plasm. Stallion semen should be cooled as soon as possible after collection to bring on quickly the state of reduced motility of sperm cells which is most necessary for preservation of their energy.

When egg yolk dilutor is used, the cooling process can be very rapid. The bottle containing diluted semen can be placed in a refrigerator or into a thermosflask containing ice-water at a temperature of 5 to 10°C.

Dorotte (1955) found the following diluent to be most effective.

Glucose ... ..	30 g.
Lactose ... ..	20 g.
Sodium Potassium tartrate ... ..	10 g.
Distilled water ... ..	1000 ml.
Fresh egg yolk ... ..	200 g.
Para amino benzoic acid	6 g.

The egg yolk is freed of abumin membrane and chalazae. The dilution rate varied from 1:3 to 1:5. Fairly good results can be obtained even after 48 hours storage and long transport.

Hejzlar (1957) claimed good results by using following diluent.

Glucose (unhydrous) ...	5.76 g.
Sodium Potassium tartrate ... ..	0.67 g.
Distilled water ... ..	100 ml.
Fresh egg yolk ... ..	3.0—5.0 g.

Dilution suggested is 1:2. With higher dilution rate the motility of the spermatozoa drops proportionately. Vlachos (1960) reported that a diluent consisting of one part of egg yolk and five parts of a solution containing 1.3% sodium bicarbonate and 5% glucose as satisfactory. Despite storage of 99 hours and agitation during transport by road the level of fertility obtained with this diluent was 5% higher than with natural service, although the conception rate averaged only 35%. Nishikawa (1959) claims good results with the following extender:

Distilled water ... ..	100 ml.
Potassium Chloride ... ..	0.08 g.
Sodium hydrogen phosphate ... ..	0.05 g.

To 95 ml. for the above solution are added.

Glucose ... ..	5.0 g.
Fresh egg yolk ... ..	3.0 ml.

6-8 ml. of semen are poured into tubes and 1-2 ml. of the above diluter termed as Baken No. 1 is added. The diluted semen is then centrifused at 1,000 to 1,500 r.p.m. for 15 minutes. The plasma in the upper layers is removed and a further 1-2 ml. of the diluent is added and then stored at 4°C. Motility of stallion spermatozoa is found better after 24 hours preservation on dilution and is satisfactory at 48 hours.

Kamenev (1955) claimed good results by using mare's milk heated to 30°C and cooled before use. Mihailov (1956) observed that mares milk was better for

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resulted in the greatest number of available spermatozoa. The first ejaculate after several weeks rest may contain large proportion of dead spermatozoa and thereafter the semen sample should be discarded. The semen from stallion and jacks is much thinner as compared to that of the bulls. The sperm concentration varies between 30 to 800 millions sperm per ml. depending on the individual capacity, feeding standard and the regime of service. Ejaculates with a concentration of less than 1,00,000 spermatozoa per ml. are not suitable for artificial insemination. The proportion of morphologically abnormal sperms even in highly fertile stallions and jacks is rather high (300 per 1000 sperm) which in bulls and rams would indicate poor fertility. Spermatozoa from stallion and jack have low viability particularly during hot season and even with a good diluent, the fertilizing capacity lasts for a couple of days only.

### Insemination Technique for the mare and jennet

Mare or jennet has to be secured properly before insemination. Restraint can be done by hobbles or by breeding chute or by backing the mare against a teasing pole. The tail is wrapped by bandage or cloth and held to one side. Area around vulval region is cleaned with soap and water and dried.

The operator should wear protective clothing and full rubber gloves. This will safeguard against spread of any infection from farm to farm. Scooping of vaginal passage by hand before insemination is not necessary. Insemination is done by means of a catheter. It is guided by introducing the hand into the vagina and the catheter is pushed into the cervix guided by the index

finger. The catheter is then directed into the uterus and insemination done by syringing with either neat or diluted semen. The suitable dose of extended semen for insemination is about 10 to 20 ml. (Fig. 152b).

For the jennet similar technique of insemination is recommended as in the mare. In the jennet the cervix is longer and more tortuous than in the mares. As such difficulty is usually experienced in the introduction of inseminating catheter into the uterus.

Inseminating mares close to ovulation time is a difficult problem, as such repeated inseminations may be necessary.

### Breeding season in the mares

The Breeding season is influenced by the seasonal factors such as the favourable times of the year when young ones can have ample to eat under domestic condition. In moderate climate there is no definite breeding season. However, seasonal effects are noticeable on the degree of their reproductive performance depending on the impact of nutritional status, extremes of temperature variations and daylight intensity. Mares are long day breeders. Seasonal influences have a marked effect on the heat period, oestrus cycle and the breeding efficiency. The normal duration of heat period extends from 3 to 8 days and most of the periods fall into the range of 5 to 8 days. A very small proportion of the heat periods last for less than 3 to 4 days. In cold countries breeding rhythm during winter becomes extremely irregular. Many mares remain in heat for a long time and others fail to come in heat at regular intervals. Better conception rates are only obtained when the heat periods show normal range and cycles become more regular.

dilution as compared to milk from other species. Better sperm survival rate was claimed in milk extender with 1:4 to 1:25 dilution rate and foaling rate there of varied from 73.5% to 93.7%.

Results obtained on various dilutors by Vlachos (1960) are as follows:

	Yolk bicarbonate glucose	Skim milk	Natural serum
No. of mares	1674	700	77
Conception rate	30%	33.9%	26%

Pernikov and Skatkin (1955) found 10% solution of honey in distilled water as a satisfactory diluent for stallion semen. Rasbech (1959) suggested use of homogenised sterilised cream as a diluent. Bunko Rogalevic (1949) observed that motility of spermatozoa was preserved for 8 to 13 days in egg yolk glucose dilutor at 0°C. The egg yolk concentration was 0.6 — 0.8% and the best ratio of semen to diluent was 1:2 to 1:3. As time progressed there was fall in the fertility rate. With certain samples semen stored upto 72 hours showed equal rate of fertility as with fresh semen. Dorotte (1955) made similar observations.

Schmidt (1950) observed that the best method for preserving semen was to keep it undiluted at 15°C with precaution not to expose it to light. Kuhr (1957) found that undiluted semen retained its motility for 82 hours and that motility was retained for 100.8 hours in

7% glucose diluent, in mares' milk for 108 hours, in 7% glucose and 5% egg yolk for 290 hours and mares' milk plus 5% egg yolk for 302.4 hours. By using the milk egg yolk glucose diluent stored for 48 hours he observed that the percentage conception rate dropped to 30% when diluted semen was required to be transported. Dorotte (1955) did not encounter pronounced decline in fertility on transport of stallion semen.

Egg yolk phosphate dilutor is not satisfactory for extending stallion semen. Adequate amounts of egg yolk and glucose are more important than the buffers in a diluent. The diluent made up of equal parts of egg yolk and 5% glucose solution shows better sperm survival than the buffered egg yolk dilutors.

About 100 to 200 units of the antibiotic per ml of dilutor exert anti bacterial effect on stallion semen without causing any damage of the sperm.

Szumowski (1954) obtained survival rates of 50% for semen diluted in glycerol egg yolk glucose with streptomycin and stored at low temperature of -79°C for 1 month.

Iljinski (1950) adopted the same technique for preservation of equine spermatozoa in yolk glucose glycerol diluent at -70°C as followed with bovine spermatozoa. After storage for 4½ months 2/3rd of the samples regained 80 to 100% motility and 1/3 of 50-60%.

Pickett *et al* (1974) observed that an average of 450 million spermatozoa were lost when semen was collected daily and well over a billion were lost when semen was collected every other day. They further concluded that a collection frequency of every other day

ing, ear back, squealing, fence pushing and striking as the most useful combination of responses for prediction of oestrus.

### Best time to breed the mares

Best time to breed a mare is near ovulation time which can only be ascertained by palpation of ovaries per rectum but this is not possible in each and every case. Riped follicles can be palpated by experienced and trained persons. The size and consistency of the ovary and follicles differ greatly from mare to mare even during the stage of oestrus.

The manifestation of various symptoms of oestrus becomes intensified with the progressive maturation of the Graafian follicle and symptoms become highly intensified close to ovulation time. At the onset of oestrus, mare shows little interest in the stallion but when she approaches the ovulation time she becomes greatly interested.

The vaginal discharge of the mare at the time of onset of oestrus is stringy and glairy. At the peak of oestrus, and close to ovulation time it is profuse and of liquid consistency. It may get accumulated in the vagina and be voided frequently. Following ovulation oestrus symptoms subside and mucus discharge becomes concentrated, sticky and grayish milky. Finally after a day or so it becomes gummy and sticks to the vagina. In a mare which is not in heat the vaginal mucous membrane is pale, sticky and rough to the touch. It is difficult to introduce hand in the vaginal passage at this stage. With the onset of oestrus the hand can be easily introduced due to the slippery mucus and the feel is smooth and velvety. During anoestrus condition the cervix is tightly closed. During earlier stages of oestrus

the cervix is partially relaxed, near ovulation time it becomes completely relaxed, smooth and tonic with rhythmic relaxation and contraction on palpation. This facilitates the easy transport of semen from cervix upwards during mating.

If ovaries are palpated at the height of oestrus large ripe follicle which feels like a blister well elevated on the ovary, can be felt. The wall of the follicle becomes very thin at the height of oestrus.

The period of heat with intense symptoms of oestrus may vary and persist for several days. It is therefore advisable that the mare which persists in heat two days after the last service should be bred again.

Some mares in heat evince heat symptoms in the presence of other mares at paddock and not to stallions. A few mares even though cycling normally fail to evince interest with males and other mares.

In the Jennet the same pattern and oestral changes are observed as in the mare except the peculiar chewing movements which are markedly noticed in the Jennet homo-sexual behaviour is also noticed which is in contrast to the symptoms in the mare. Jennet in heat will ride other Jennets or will stand to be mounted. Intensity of heat in Jennet may vary due to various factors and as such it is better to check up with the help of a teaser.

### Breeding at Foal heat

The first heat after parturition is called foal heat and may occur 3 to 4 days after foaling or as late as eleven days or even more. The duration of foal heat is the same as heats during other periods. It is usually presumed by breeders that



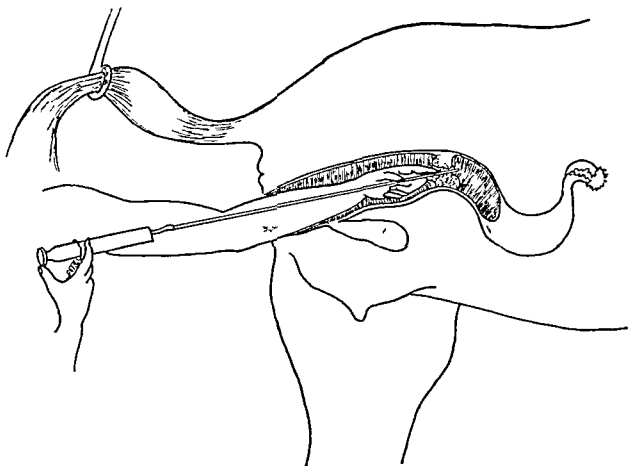


Fig 152(b) A.I. Technique in mare.

It is customary to tease the brood mares about 18 days after the cessation of oestrus to confirm if they are pregnant. However great variations are noticed in the interval between two heat periods due to influence of variety of factors. Mares with continuous oestrus and those with absence of external symptoms will also be cycling normally with regards to the formation of follicle and fertilizable ovum. It is desirable to keep the pregnant mares under close observations throughout the breeding season on account of the fact that the pregnancy may be interrupted by abortion or embryonic death. Such mares may resume their heat cycles.

Jennets more or less behave similar to the mare with regard to their breeding habits except that a few may show

greater irregularities. Length of heat period ranges from 3 to 8 days as in mares. In unthrifty jennets it is slightly longer. In contrast with the mare they may show short oestrus period of only 1-2 days in fall and not during summer. In consequence of this some jennets settle easily with very few services during the fall than during the spring and summer. Back *et al* (1971) reported the mean duration of oestrus, diestrus and oestrous cycle in mares as  $6.8 \pm 2.3$ ,  $15.0 \pm 2.1$  and  $21.5 \pm 2.5$  days respectively. The range in duration of oestrus, diestrus and oestrous cycle as observed by them was 2 to 24, 5 to 21 and 13 to 30 days respectively. They recorded 9 criteria — eversion of vulvar labia (winking), tail raising, squatting, urinating, kick-

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with cessation of heat two days after last service the chances of conception are fairly good. This is possible when parturition has been normal with no retained placenta, uterus is healthy, free from infection and involution complete. On account of uncertainty of these requirements at foal heat, many breeders prefer to breed at the next heat period which occurs at about 30 days after foaling.

### Factors affecting semen production in the Stallion

Production of semen from stallion and jacks differ greatly from that of bulls and rams. In many respects it is similar to that of the boar. Both stallions and jacks discharge large volumes of ejaculates varying between 25 to 150 ml. in some individuals, as much as 400 ml. Stallions of warm blooded breeds give small ejaculates with high sperm concentration as compared to the voluminous ejaculates obtained from draft stallions and jacks. After prolonged sexual rest the first ejaculate is usually large. With frequent services the later ejaculates become smaller in volume and the quality of semen drops very rapidly as compared to that in the bulls and rams. Stallions of high vigour can give two services a day, but the period for which they can extend such services is very short. One service a day throughout the breeding season ensures fairly good conception rate. In addition to the season, feeding has a significant influence on the semen production. Unthrifty stallions produce poor quality of semen. Provision of green grass, oats and other concentrates is most essential during breeding season for the production of high quality semen.

Several factors contribute in causing subfertility or temporary infertility. These may be physiological, pathological or psychological. Insufficient exercise, overwork or poor condition are some of the common causes. Caljuk and Rumanceva (1958) suggested feeding of increased protein rations even fish meal to stallions in production. Skatkin (1951) observed that when stallions were fed concentrates such as blood, milk and bone meal and used successively for a week, there was decrease in the volume of semen and increase in sperm motility. Rations mainly composed of hay, oats and bran alone caused deterioration in the quality and quantity of semen. Higher plane of nutrition, improved fertility in young stallions than in aged stallions above 8 years of age. Seidler and Zolkiewski (1951) reported that increase of oats in the ration had a beneficial effect on production of semen. Syresin (1953) reported that replacement of some of the grains by yeast led to an increase in the volume of semen but it did not significantly improve the fertilizing ability. Sinjaeva (1951) failed to record any difference in concentration or volume of semen in different diet groups, survival of spermatozoa however was markedly better in stallions fed with experimental ration.

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# Chapter 43

## Artificial Insemination in the Sheep

During the recent years, it has become necessary to breed sheep by artificial insemination method for making intensive use of superior sires. Russia is the foremost country in the world where thousands of sheep are annually bred by artificial insemination. Kenya, South Africa, Rumania, Australia and Argentina are other countries where limited use of this method of breeding is being done in selected flocks. In India, it has been tried with success on sheep breeding farm at Poona by Sane *et al* (1952), at I.V.R.I. by Shukla & Bhattacharya (1952) and by Roy and his colleagues (1956) at Mathura on experi-

In flocks where it is not possible to settle the ewes in good time, naturally it results in a prolonged lambing season with the consequent lowering of uniformity of the lamb crop. This method of breeding is very expensive but considering the mass scale development in economic characters, adoption is most essential. The method may prove economical in case large number of flocks where grading is desirable are concentrated in a locality and where there is dearth of quality rams for breeding purposes.

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Limited use should be done in case rams are not progeny tested for fear of transmission of undesirable traits especially lethal and sub-lethal characters. It is desirable to use rams with very high fertility rate so that the flocks can be settled in a reasonable period of about 60 days under natural mating.

### Management of Rams

Ram sperm takes 40 days to mature. The sperm cells which are required for A.I. programme are developed during two months prior to their collection.

Most of the inseminating programmes are carried out from mid February till mid May, as this is the period when ewes are normally cycling. However, most of the semiarid areas experience very hot summers, resulting in summer infertility.

Rams, should have access to pasture or irrigated plots and before they are let out to pasture, should be inspected daily for fly strike, screw worm and foot

abscess. Two tooth rams are segregated from adults to prevent fighting and homosexuality.

When the actual inseminating programme starts, the rams are still housed and fed as before and walked to the shearing shed and back daily, to ensure exercise. When in use at the shearing shed, they are penned separately awaiting their turn as semen donors to avoid fighting with other mates and to save time in handling.

The training actually does not begin till the programme starts and it seldom takes more than two days to get all rams to serve a ewe in a bail and subsequently into an A.V. The number of collections to be obtained in a day varies from ram to ram. From most of the rams semen can be collected three times a day producing average ejaculates of 1.0 ml., sperm concentration of  $35 \times 10^6$  per ml. live counts 85-90% (Miller, 1975). He further reported  $15,000 \times 10^6$  sperm per day from two tooth rams in three collections.

Some typical results of rams semen examined on day 6th of a programme are presented below:

	Vol. (ml.)			Sperm No. $\times 10^6$			Live (%)		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Ram A—3 Yr.	0.7	1.4	0.8	39	45	35	90	92	88
Ram B-3 Yr.	1.0	1.0	1.1	41	31	31	90	86	88
Ram C—1½ Yr.	0.8	0.6	0.5	34	45	45	82	95	95
Ram D—1½ Yr.	0.7	1.0	0.4	54	43	36	95	95	95

The semen from the same rams was collected thrice daily and on day 15th the collections were examined.

	Vol. (ml.)			Sperm No. $\times 10^6$			Live (%)		
	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3	No. 1	No. 2	No. 3
Ram A	0.6	1.0	0.8	40	40	35	90	90	90
Ram B	1.0	1.0	1.0	40	35	30	90	85	90
Ram C	0.0	0.6	0.4	32	40	15	80	90	95
Ram D	0.7	0.5	0.7	45	40	35	95	95	90

50% of these were abnormal. During the months of July to September, there was complete cessation of spermatogenic activity. The semen was devoid of spermatozoa, ejaculates were watery and resembled those obtained from vasectomised rams. The restoration of normal spermatogenesis started from the end of October. There was remarkable improvement in November. The average initial motility was 3.2; the percentage of abnormal spermatozoa came down to 10.6% and the semen quality was excellent in December and January as in indigenous rams. The semen picture was normal and the abnormal sperm count was only 3 to 4%. These variations in semen quality are attributed to the prevailing ambient temperatures. Decline in spermatogenesis was apparent when environmental temperatures increased beyond 80%.

It is evident that there is periodic rhythm for spermatogenesis. This pattern is influenced mainly by high temperature leading to deteriorating changes in semen quality which usually start from the mid-summer. The semen quality gradually improves by mid-autumn. It is not clearly known to what extent the following factors influence the semen production—shade available, winds, amount of wool on rams, availability of greens, feed, short term heat waves in some summers and not in others and frequency of service and/or collections.

Decline in semen quality should invariably be accompanied by a gross reduction in the initial motility, sperm count and increase in the proportion of abnormal spermatozoa. It is on the basis of such changes in semen pictures of rams in areas away from the equator that a gross decline in semen quality has been reported by Comstock *et al* (1943).

Bogart and Mayer (1946) and Cupps *et al* (1960). Deshpande (1970) observed under Bombay climatic conditions in Bannur  $\times$  Somali rams that the best semen samples were obtained during spring and winter. During these seasons the relative humidity was lower and differences between morning and evening levels of humidity were higher (7 to 11.5%); the minimum temperature was lower and the differences between maximum and minimum temperatures were wider (12° to 13°C) and the rainfall was nil or negligible.

Studies show that shifting indigenous rams even from far distant areas to other tracts which fall within the broad range of tropics and subtropics do not impair the reproductive efficiency. Mandya rams which were brought from South to North India to an approximate distance of about 1500 km. did not alter in their reproductive efficiency. The variations in temperature or duration of daylight had no effect on the production of quality semen throughout the year (Sahni and Roy, 1969).

### Day Light

Ortavant (1958) studied interaction between temperature and photoperiodism on spermatozoal production. His p-32 tracer studies in spermatogenesis show that the rate of division of primary spermatocytes and time of subsequent maturation are relatively unaffected by the photoperiodic environment but the number of spermatids which survive the complete maturation process are affected. The failure rate was observed to be high under conditions of increasing daily illumination. He observed that the effect of high temperature in the spring and early summer months superimposed on this

suitability for use as sire. Selection for mutton characteristics can be done at an early age as growth rate is the important criteria. Watson *et al* (1956) observed that testicular development can be correlated more with live weight of the ram than with age.

### Factors affecting semen quality

#### 1. Season

Marked seasonal variations in density, percentage of abnormal sperms and glycolysis in semen of rams was observed by Comstock *et al* (1943). Poor quality of semen was recorded during summer and early autumn months i.e. July to September. Bell (1945) observed slight seasonal variations in Rambouillet rams in which semen quality was poor in spring i.e. March-April. It improved in summer (May-July). Koger (1951) found that in autumn, ram semen has higher motility and a longer viability on storage than in the semen collected in spring. Maqsood (1951) on his observations on young Sufflok rams reported that during the non-breeding season, there is a marked decrease in the libido, volume and spermatozoal density and increase in the number of abnormal sperms. Poor libido is believed to be due to interference with the production of androgens by the interstitial cells. Histological examination of testes of the young ram carried during spring and summer revealed arrested spermatogenesis in the majority of the seminiferous tubules. Watson (1952) observed that Marino rams show higher fertility in the autumn but there is uncertainty in the spring. Wiggins *et al* (1953) found a significant correlation between libido and fertility in the range rams. Volcani (1953) observed seasonal variations in spermatogenesis in Awassi sheep in Israel. In July and

August when days, are long, and hot, histological examination of testes in rams indicated active spermatogenesis, where as from September to December, degenerative changes were noticeable. He considered that the temperature was not the causative factor, and implicated availability of grazing facilities. Shukla and Bhattacharya (1952) observed that the reaction time, differed significantly between rams, but there was no seasonal trend and no relationship to quality of semen. Significant seasonal variations in the volume of semen, pH, sperm concentration, motility and percentage of abnormal spermatozoa were observed. Variations between months and within seasons were also significant. The quality of semen was poorest in autumn and best in spring. Hafez *et al* (1955) observed that the quality of semen of Ausimi and Rahamani rams in Egypt was very good during spring and autumn than in summer and winter. Hulet *et al* (1956) found that ram fertility was 42.8% before September due to poor quality of semen and thereafter the fertility improved to 67.6%. Sahani and Roy (1967) observed in their studies on Romney Marsh rams brought in Uttar Pradesh (India) for improving the indigenous sheep, that in the month of January, the abnormal sperm count was 3.8% but during the month of February, as the climate begins to get warmer, the percentage of abnormal spermatozoa increased to 27.5%. The signs of seminal degeneration gradually developed, thereafter as the summer advanced the initial motility became poor, sperm concentration decreased, more than 90% of the sperms were found dead and the number of abnormal spermatozoa also increased. In the month of June, the wave motion was completely absent, all spermatozoa were dead and nearly



## Shock

Webster (1952) observed that in Newzealand, a cold southerly wind during night rendered 18 rams infertile over night and the condition persisted for remaining period of the breeding season. He also observed from the past record that abnormally low lambing percentage was related to a sudden exposure to stormy weather during the mating season.

Dun (1956) recorded temporary infertility in rams, due to damp and marshy conditions which led to seminal degeneration associated with flabbiness of the testis. The condition was accompanied by warm humid weather and mosquito plague, shortly before mating.

## Feeding of Thyroproteins

Feeding thyroproteins has not produced beneficial results in rectifying the seasonal effect on rams semen.

## METHOD OF SEMEN COLLECTION IN THE RAM

There are three methods of collection  
(1) Anoestrus ewe (2) Artificial vagina  
(3) Electrical stimulation.

### Anoestrus Ewe

In this method the rams are trained to serve anoestrus ewes and after the service the semen is aspirated from the vagina. It is unsafe to use the semen so collected for fear of contamination.

### Artificial vagina

The pattern of A.V. is similar to that in the cattle, except that the A.V. is short. The outer hard plastic casing is  $15 \times 5$  cm. and the inner liner is  $25 \times 3.75$  cm. The A.V. is prepared in the usual way by pouring hot water

( $45^{\circ}\text{C}$ ) through the inlet on the outer jacket. Necessary air is blown to provide cushioning effect and air valve tightened. Both the temperature and pressure are important. When the A.V. is ready, the inner temperature should be  $40^{\circ}\text{C}$  (Fig. 153a).

Deshpande (1970) from his experience of collecting semen of rams considers that, it is advisable to warm the A.V. first with hot water ( $47^{\circ} - 50^{\circ}\text{C}$ ) initially and then pour out and refill the A.V. with hot water at  $45^{\circ}$  to  $47^{\circ}\text{C}$ , depending upon the season, in order to maintain the inner tube temperature between  $41^{\circ} - 45^{\circ}\text{C}$  at the time of collection.

A rubber cone is attached to one end of the A.V. at the end of which a graduated 5 ml. test tube is fitted to serve as a receptacle for the semen. The open end of the A.V. should be properly lubricated preferably with sterilised white vaseline. A glass collection cup is now directly fixed to the other end of the A.V. to obtain direct collection (Fig. 153b). Rams are very sensitive to temperature variations and therefore adjusting proper temperature in the A.V. is important. Once the rams are exposed either to an A.V. which is too hot or cold, they may dislike or refuse to serve on subsequent occasions. A freshly prepared artificial vagina should be used for each collection from a ram to avoid contamination. It should be properly sterilised after the use and kept in dry sterile chamber ready for use.

### Electrical stimulation

The equipment for collection of semen from the rams by electrical stimulation as outlined by Dunk et al (1954) is said to be satisfactory. The power is obtained from 110 volt battery

basic photoperiodic effect is to destroy a high proportion of the relatively few spermatozoa which have survived the maturation process. Deshpande (1970) observed that during spring and winter under Bombay climatic conditions, the day length varied between 11.02 and 12.56 hours, whereas, the actual day light hours varied from 7.7 to 9.8 hours and the semen quality was best during this season.

### Breed

Very little studies have been done on breed differences in semen production and on the interaction between breeds, season and temperature. Gunn *et al* (1942) observed that British breeds in Australia are susceptible to the high environmental temperatures similar to Merinos. However, the period of minimum spermatogenesis did not correspond with the high environmental temperature of mid and late summers but were observed in spring. Under Indian conditions autumn appears to be the season characterised with poor quality of ejaculates and summer provides most superior quality of semen, as has been reported in the indigenous breeds like Bikaneri and Mandya (Shukla and Bhattacharya, 1952; Sahni and Roy, 1969; Deshpande, 1970). This is in contradiction to the general trend in temperate breeds reared under temperate or tropical conditions.

Singh and Roy (1963) in their studies on Romney Marsh rams (U.P.) observed that similar to Bikaneri and Mandya breeds, the autumn season is characterised by poor quality semen but unlike these breeds which produce best semen in summer, it is in winter that the semen of Romney Marsh rams has the

best quality. As against this, Emmens and Robinson (1962) observed that Romney Marsh rams give most satisfactory semen in autumn in Australia. It is due to such differences in response of different breeds to different seasons that Webster (1952), Mies Filho and De Almeida Ramos (1956b) suggested that extreme care is required in choosing rams of right breed for a particular environment. It is not clear whether such limited data is adequate to prove breeds and environment interactions i.e. differences in sensitivity of breeds to environmental conditions, but gives a sufficient caution before any large scale importation of exotic sires is made with an aim to improve the local breeds.

### Altitude

High altitude and poor nutrition are deleterious to fertility in rams (Easley, 1951).

### Disease

Any infection such as foot rot or abscesses which might cause general febrile condition will result in seminal degeneration (Gunn *et al* 1942). Morley (1948) reported that scrotal fly strike may not necessarily impair fertility. Engela (1918) reported incidence of epididymitis, varicocele, scrotal tumours and other diseases of male genitalia. Miller and Maule (1954) observed 6.3% of the above conditions in 16,655 Merino rams examined clinically. Andreevskii (1940) observed that the main cause of semen abnormality in 40,976 adult rams was chronic orchido-epididymitis. Of these about 15% of the rams showed morbid and degenerative changes in the testes, mostly in epididymis.



FIG. 1210. Material from the



FIG. 1211. Condition of the material  
at Meridien



FIG. 1212. Condition of the material  
at Meridien

with 60 cycles which is reduced by transformer to 30 volts. This isolates the electrodes from the line of source. The probe for insertion into rectum is made up of rubber hose about  $3/4$  inch diameter with solder rings one inch apart. Alternate rings or electrodes are connected to provide the difference in electrical potential between adjacent electrodes. With this equipment the collection can be made in standing position. However, at times it is necessary to restrain the animal in an extended position on a table. The rectal probe is lubricated, moistened and inserted so that the last ring is located just inside the anus. The penis may be gently pulled out before stimulation. But on many occasions it generally protrudes with stimulation. The end of the penis should be held in a glass vial or a test tube. During the process the voltage is gradually increased and then reduced to zero during a five seconds period followed by an equal period of rest. Stimulus is gradually increased until the semen is ejaculated in the order of 2, 5 and 8 volts. The number of stimuli necessary to produce semen is variable.

#### **Merits and Demerits of different methods of semen collection**

Collection of semen by the A.V. is considered as the best method. Collection by electrical stimulation has an advantage that training of the rams is not necessary. When a large number of rams are to be tested for semen quality, collection by electrical stimulation is advantageous. The major disadvantages in the electrical stimulation is the likely injury to the animal which can be minimised by the use of the bipolar electrode. Other disadvantages are (1) slightly lowered spermatozoal density, (2) the possibility of contamination

of semen by urine, (3) difficulty in obtaining second collection within short time if the first one is not satisfactory and (4) the refractoriness on the part of some rams.

Experience shows that by collection in artificial vagina the volume of one ejaculate is smaller but denser as compared to an individual collection by electrical method. A series of collections can be made in an artificial vagina which is *not possible by the electrical method*. High quality of ejaculates is the main advantage in collection of semen by artificial vagina.

#### **Frequency of collection**

Rams are peculiar in their capacity for frequency of ejaculates with optimum quality. Ejaculates to the extent of even 20 to 30 a day followed by successive collections of equal number of ejaculates the next day have been obtained. Collections to the extent of 2 to 6 per day even have been obtained over a period of a week or 10 days (Chang, 1945). Salamon and Robinson (1962) observed that season and nutrition did not measurably affect frequency of service nor there was any apparent effect of season on volume, density or sperm count. However, marked effect was seen on these characters due to high protein ration. Satisfactory fertility can be maintained even with frequent ejaculations if the total number of spermatozoa is more than  $80 \times 10^6$ . Fall in lambing rate is observed when the sperm count is reduced.

#### **EVALUATION OF SEMEN**

##### **Colour and consistency**

The colour of ram semen is creamy. Variable grades are noticed according to frequency, season, age, rest and amongst

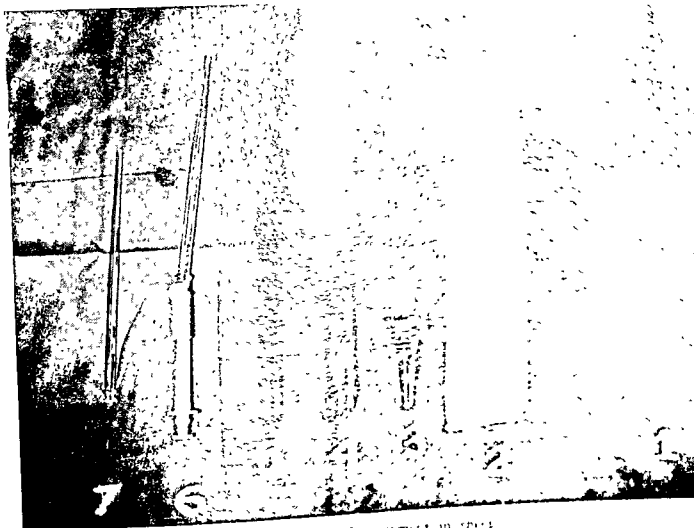


Fig. 153(a). AI equipment in 1964



Fig. 153b. Collecting of water from a well in 1964



individuals. The normal consistency is thick which is however variable. It may be classified as dense, medium and thin. It appears that there is very little direct evidence about the value of density and motility determinations in ram semen.

### Volume

Considering the large size of the testicle in the ram, the volume of semen obtained at one ejaculate is relatively small on an average from 0.5 to 2.0 ml. Wiggins *et al* (1953) observed that, the volume of ejaculates gives a low but significant positive correlation with fertility.

### Mass Activity

When a fresh drop of ram semen is examined under the microscope at body temperature, very brisk dark grey whirls are noticed. The swirling motion is very rapid and dense in appearance. The grading is done as for bull semen.

### Initial Motility

As in case of bull semen, a drop of semen is diluted in Ringer's or Sodium citrate solution and examined under high power. Gradation of motility is done as for bull semen.

### pH

The pH of ram semen varies between 5.9 to 7.3, with an average of 6.4 (Emmens, 1959).

### Sperm concentration

The average sperm concentration in a single ejaculate of ram semen contains on an average 2 to 3 billion spermatozoa per ml. In a good sample about 90% are alive. Ram semen with high sperm concentration is slightly acidic in re-

action, while, with low sperm concentration it is slightly alkaline. Semen with alkaline reaction is often associated with poor quality and low fertility (Anderson, 1945).

### Morphology of ram spermatozoa

A normal ejaculate may contain 5 to 15% of abnormal spermatozoa. A greater proportion of abnormal spermatozoa may lead to poor fertility. The most common forms of the abnormal type are tailless and deformed. Other morphological abnormalities found are tapering heads, enlarged middle pieces and adhered, coiled or bent tails. It is usually observed that head abnormalities cause low fertility. The types of morphological abnormalities are of more importance than the total sperm count. High percentage of pyriforms or narrow heads are particularly associated with poor fertility.

Ram semen with sperm concentration of less than one billion per ml. with a pH of 8.00 or higher, with slow sluggish motility, and with more than 25% abnormal type and more than 1% abnormal sperm heads are of doubtful fertility and should not therefore be used for insemination.

### Percentage of live spermatozoa

The percentage of living sperm may be estimated by the vital stains (Lasley *et al*, 1942) or by direct counting. A stain containing congo red and nigrosin gives satisfactory results for ram semen and it is preferred to Eosin and nigrosin stain (Blackshaw, 1958b). The percentage of live sperm is not significantly correlated with fertility in a ram but the percentage of live normal spermatozoa has a significant correlation (Wiggins *et al*, 1953).

### Biochemical studies of ram semen

In the ram semen, 1/3 of the portion is made up of spermatozoa and 2/3 by the seminal plasma. There is marked difference in composition between the sperm and the seminal plasma. Nucleic acid is confined to spermatozoa. The spermatozoa contain more of iron, zinc, copper, haematin and chiefly as cytochrome and plasmalogen than the

<sup>1</sup> plasma. Acid soluble phospho compounds are present in the sperm <sup>2</sup> also in the seminal plasma. Fructose and citric acid are present in large concentrations in ram semen and these are derived from the seminal vesicle secretions (Mann 1964).

Table 58  
RAM SEMEN MAIN COMPONENTS  
(Mann 1964)

(Results are average values expressed, unless otherwise stated, in mg/100 ml. With the exception of the uric acid (Leone, 1953b) and CO<sub>2</sub> content (Shergin, 1935) the data are our own, based on analysis of material pooled from ejaculates of ten rams, average volume of single 1.2 ml, average density 2,940,000 sperm per ml.)

Dry weight	14,820
Chloride (cl)	87
Sodium	103
Potassium	71
Calcium	9
Magnesium	3
Inorganic phosphorus	12
Total nitrogen	875
Non protein nitrogen	57
Urea	44
Uric acid	11
Ammonia	2
Fructose	247
Lactic acid	36
Citric acid	137
CO <sub>2</sub> content (ml /100ml)	16
Ascorbic acid	5

### Diluents for ram semen

The egg yolk diluent developed by Laidy and Phillips (1939) is found satisfactory for ram semen. For this purpose, equal volumes of fresh egg yolk and sterilised phosphate buffer, (0.2 gm Dihydrogen potassium phosphate and 2.0 gm, D<sub>1</sub> sodium hydrogen phosphate per 100 ml of pyrogen free triple distilled water) are mixed thoroughly. The pH of the mixture is close to 6.75. If it is not, it should be adjusted by the addition of sterile M/15 citrate buffer or bicarbonate can be used to replace the phosphate buffer without effect on the glycolysis (Moore 1949). The replacement of the citrate buffer with glycine has improved the survival time of ram semen (Ahmed, 1955; Roy *et al*, 1956 and Skoloskaya *et al*, 1956).

Milk appears to be the most useful diluent for ram semen (Istvon, 1956; Filimon *et al*, 1956; Macpherson, 1957 and Hill *et al*, 1958). Miller (1975) reported the use of diluents like egg yolk citrate, heated milk from ewes, cow skim milk and accessory secretions from vasectomised rams. Of the above diluents cows milk or skim milk heated at 92°C for 10 minutes found to give good result with dilution rate 1:1.

Kuznetsov (1934) recommended that at least 50 million spermatozoa per dose are necessary for conception in case the diluted semen is deposited in the cervix proper.

### Storage and transport of semen

Yoshioka *et al* (1951) reported that ram semen can be successfully stored for seven days with high fertility rate. Temperature at 0°C is reported to be optimum for storage of ram semen (Moore *et al*, 1940). Storage of ram semen at 20°C in the presence of CO<sub>2</sub>,

is also considered as good (Blackshaw, 1958b). Sudden temperature changes are considered harmful for the viability of the sperm (Chang and Walton, 1940). Semen should be cooled gradually for storage. Blackshaw (1958b) reported that the presence of egg yolk in the diluent is largely responsible for preventing the cold shock.

The metabolism of ram semen is mainly glycolytic (Mann, 1964). Salisbury (1946) reported that since the metabolism of ram semen is mainly glycolytic, oxygen may damage the spermatozoa. Complete anaerobic condition is therefore necessary for storage. The viability of ram semen is prolonged with effective exclusion of air during storage. It is preferable to seal the ampoules with minimum air space as far as possible to avoid agitation during transport.

### Insemination technique

Under the insemination technique there are a number of pre-requisites such as equipment for insemination, restraint of ewes, number of ewes for insemination and adjustment of inseminations during better part of oestrous period and whether heterospermic inseminations are desirable.

### Equipment

A speculum, a head light torch and a syringe with insemination pipette is all the required equipment. There are three types of specula in use (1) the duck bill type (2) metal barrel (3) cold lite plastic with attached light. It is the usual experience that the duck bill type is easy for insertion and gives greater ease and freedom of movement. It can be easily dilated in the vaginal passage and removed in a closed condi-

tion after use. It is also easy for sterilization. A glass speculum is also conveniently used.

A 2 ml. glass syringe attached to inseminating pipette with a rubber connection is most convenient for insemination. A fresh inseminating pipette should be used for each insemination (Fig. 153a).

### Restraint of Ewes for Insemination

With the help of two attendants, ewes can be inseminated at the rate of 100 an hour, if the operation is streamlined, using fresh neat semen. Various methods of holding ewes have been used and some methods used in Australia between 1950 and 1960 are:

1. Ewes held upside down in a cradle.
2. Ewes placed on a battery of bails on a raised platform.
3. Ewes held on a rail, as for lamb marking.
4. The operator working in a pit and a ewe manoeuvred upto a hock bar at the edge of it.

Other methods used in Asia and in South America, include the use of various cradle and jacking devices to lift the hind quarters. These are not easy to operate. The method used in Australia, at present, is for the attendant to straddle the ewe facing the tail and with a hand under each flank to throw her hind-quarters over a rail 24" above floor level. Attendant keeps the ewe's hind legs extended by pressing into her stifles. Provided the ewe's front feet are on the floor, she will not struggle. After insemination the ewe is either released through a gate in the pen or a coloured plastic peg put into the fleece and released into the mob.



Table 59

## PERCENTAGE OF EWES IN HEAT ACCORDING TO DURATION OF OESTRUS

Breed	12-24 hrs.	24-48 hrs.	48-72 hrs.	72 hrs. & above
Jaisalmeri	45.30	40.00	13.00	1.80
Malpura	28.00	52.50	16.00	2.50
Chokla	20.60	49.00	31.70	4.70
Rambouillet × Chokla	40.80	40.30	12.50	1.80
Rambouillet × Malpura	36.60	45.80	13.70	3.60
Rambouillet	43.20	15.40	9.56	1.89

(Tiwari *et al.*, 1973)

A false floor about 2 feet high is put into the catching pen to enable the inseminator to have the ewes presented at eye level.

When the ewe is placed over the rail, an assistant inserts a glass speculum whilst the inseminator loads his syringe. The inseminator then holds the speculum, locates and positions the cervical opening and inseminates the ewe. The semen is deposited in the cervix (Fig. 153c).

A simple wooden crate with sloping platform is considered for restraint. The rear portion is raised to a height of 3 feet so that the inseminator can conveniently inseminate in standing position (Sane *et al.* 1952).

#### Time of Insemination

During oestrus, mucus flows freely from the cervix into the vagina. Generally there is a small amount of clear mucus at about the time oestrus commences, but as oestrus advances the amount of mucus increases and its nature changes. First it becomes cloudy, then creamy and finally cheesy in consistency. Although it is usually considered that changes occur progressively as

oestrus advances, these cannot be used to time different stages precisely. However Morratt and Dun (1960) have demonstrated an association between fertility and the different types of mucus at the time of insemination (Table 59 and 60).

Table 60  
THE FERTILITY OF EWES WITH  
DIFFERENT TYPES OF VAGINAL MUCUS

Type of mucus	Grade	No. of ewes inseminated	Fertility %
Clear and sparse	1	113	50
Clear and copious	2	369	62
Cloudy and copious	3	219	62.11
Cloudy	4	102	52
Creamy	5	92	33.33
Cheesy	6	34	15

Uterine contractions and motility of the spermatozoa are largely responsible for quick transport of spermatozoa through the tubular genitalia to the site of fertilization in about 2 to 4 minutes. The ova are reported to survive from 12 to 23 hours in the fallopian tubes but it is not very clear if they are fertilizable during this period. Generally, 8 to

10 hours appears to be a reasonable time for the survival of tubal ova. Therefore, inseminations done any time within the oestrous period may result in fertilization with the greater possibility of success to insemination done during mid-oestrus and least possibility in the later part of oestrus i.e. close to ovulation time.

Sinclair (1957) confirmed this concept based on his studies on 1500 Merino ewes bred naturally or by artificial insemination in early, mid or late oestrous. Conception by natural mating at each stage resulted in 55-65% fertility. Lower conception rate resulted due to inseminations in late oestrus when the cervical mucus was becoming creamy. The observations of Carbonero Bravo (1955) show that the optimum time of insemination was mid oestrus i.e. 12 to 18 hours after onset of heat. Osikoski (1956) obtained highest lambing percentage 12 hours after the onset of oestrus even by natural service in 175 Mountain ewes and 8 hours after in 122 Merino ewes. There was no difference in the conception rate of ewes (53.2%) between morning and evening inseminations based on 45 day non returns (Mies Filho and De-Almeida Ramos, 1955a).

#### Seasonal influence on successful percentage of inseminations

Season of the year has a marked influence on the successful percentage of insemination. Dun *et al* (1960) observed significant differences in favour of inseminations done during autumn season. Salamon and Robinson (1962) observed 20-25% more of lambing crop in ewes inseminated during autumn as compared to those inseminated in spring. The difference is not due to differences in semen quality. Ewes re-

turning to service during autumn is an evidence of reliable estimates of conception rates. On the other hand, percentage of returns in the spring can not be relied upon for a true estimate. Difference in the percentages of lambing crop are apparently visible between autumn and spring. Salamon and Robinson (1962) observed that of the estimations done on the sum of returns to service, 92% of successful inseminations were accounted for in ewes during autumn to a single insemination as compared to 71% in the spring.

#### Number of Inseminations

Question arises as to the number of inseminations necessary to settle the ewes in the same oestrus. Beneficial results have been observed by various workers and they claim an increase in conception rate from 4 to 14 percent by doing insemination per oestrous period (Glembokii and Vasiljev, 1944). It is considered advisable to do second insemination on the day following first insemination of ewe still in oestrus. Similar results have been obtained by Guitierrez-Fibera (1948) and Osikowski (1956) in Merino ewes at 24 hours intervals following natural service.

#### Volume of semen and number of spermatozoa per insemination

Kuznetsov (1934) recommended that at least 50 million sperms per insemination are necessary for conception. Experience show that dose as low as 0.1 ml. of neat semen is satisfactory for higher conception rate (Sinclair, 1957; Keast and Morley, 1949). A volume of 0.05 to 0.2 ml. is found satisfactory which is expected to contain 50 to 100 million sperms. A fairly good quality of ram semen should contain on an average about 150 million sperms per 0.05 ml.

Higher conception rates have been claimed in ewes by using neat semen as compared to the rates where diluted semen is used (Mies Filho and De Almeida Ramos, 1955-b). In general it is observed that a conception rate of about 55 to 60% can be claimed to a single insemination if a dose of 0.5 to  $1.5 \times 10^8$  spermatozoa in 0.05 to 0.1 ml. of semen, diluted or undiluted, is used for cervical insemination.

### Site of Insemination

Dun (1955) observed that the external appearance of the cervix varied a good deal and difficulties may be experienced in locating the opening to the cervix. Because of the peculiar structure of the cervix of the ewe, it is not possible to deposit semen in uterus. It may be placed (i) in the opening to the cervix, (ii) on the cervix as it projects into the anterior end of the vagina or (iii) in the anterior end of the vagina.

### Insemination

When the ewe is presented for insemination, the lips of the vulva should be cleaned by wiping them towards the anus with cotton wool soaked in normal saline. A speculum lubricated with white vaseline is slipped into the vagina with a rotary motion and the cervix is located and imprisoned in the opening of the speculum. The semen is drawn into the insemination pipette and the syringe and the tip of the insemination pipette is inserted gently into the opening of the cervix. About 0.1 ml. of semen is deposited into the opening of the cervix and then the insemination pipette and the speculum are withdrawn.

### Heterospermic Inseminations

Favourable results have been claimed and higher conception rates obtained

by use of pooled semen samples from different rams (Aslanjan and Camuha, 1950; Mamedjarov, 1952; Stojanovskaja and Mesjacev, 1957). However, similar results could not be reproduced in Australia (Dun, 1956).

### Detection of oestrus in Ewes

There are two ways of detection of oestrus in ewes. One is by hand teasing in yards with a vasectomised ram. A loose piece of soft thick cloth is tied to the girth and kept hanging in front of preputial orifice, to avoid natural service. In the second method, a vasectomised, breast painted with red clay (Geru under Indian conditions) ram is allowed to run free in the flock. The fluid paint is renewed daily. Ewes which are marked with paint are separated for insemination and next morning they are teased again to confirm if the heat is over or else a second insemination is done to those which are still in oestrus.

Ewes remain in oestrus for about 30 hours on an average. In exceptional cases, it may extend over 2-3 days. The ovulation may vary considerably in relation to the onset of heat. McKenzie and Terrill (1937) observed that ovulations in the ewe generally occur at the end of heat period. Insemination done before the end of heat period results in higher conception rates than those at or after the end of the heat period.

### Faulty techniques resulting in low fertility

Some obvious things that lead to failures are (1) Fear of not mustering ewes quickly enough so that they are kept in small paddocks and often under fed. (2) Faulty mustering — ewes in oestrus missed and not presented till 24 hours — too late. (3) Failure to look

- after rams prior to the collection time. (4) Too much time spent in drafting sheep. (5) Failure to recognise vaginal mucus changes, thus leading to faulty teasing. (6) Faulty management of Donor rams causing loss of libido. (7) Faulty management of rams e.g. mixed age groups together leading to fighting, mounting and a waste of semen. (8) Bad handling and unnecessary handling of ewes after insemination. (9) Failure to have strong and fit teasers.

### Failure in A.I. technique

- (a) Failure to control temperature of semen. (b) Failure to pick up and position cervix quickly. Glass speculum with a lip is used in order to position the cervix and get good lighting. (c) Using too much air in the syringe as an air pump. This blows the semen out of the cervix entirely. (d) Not withdrawing speculum before depositing the semen. If this is not done a negative pressure is set up and the semen is withdrawn (at times completely from vagina). (e) Lack of speed.

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# Chapter 44

## Artificial Insemination in the Goat

Artificial Insemination has proved successful in goats. In a tropical country like India where goats are kept in large numbers under village conditions, this method holds promise in the grading up programme.

Blokhuys (1962) has mentioned that in Germany there is a high incidence of sterility in male goats in consequence of which attention has been focussed to breed goats from selected sires by artificial insemination. In Netherlands and France the technique has been extensively used, with advantage.

### Collection of Semen and Artificial Vagina

The young buck should be encouraged to mount on another buck or on an anoestrus doe. If the buck refuses to mount initially, a goat on 'heat' may be used for collection. Collection of semen by means of the artificial vagina is the best method. In general, sexual maturity in male goats is reached between 6 to 8 months and goats can be trained easily to serve in A.V. The artificial vagina used for bucks is similar to the one used for rams. The artificial vagina consists of a cylinder and a rubber liner which is inserted and ends are folded back over the cylinder. The space bet-

ween the rubber liner and cylinder is half filled with warm water at 50—60°C and air is then blown in through the valve to adjust the pressure. One end of the A.V. is fitted to a collecting tube and the other end is lubricated with soft paraffin. The temperature of the A.V. is 36 to 42°C. The buck semen is highly sensitive to cold shock as such the A.V. when ready should be wrapped in a towel.

### Frequency of Collection

This depends largely on the individual, breed and age of the animal. As a standard one collection daily can be the practice. However, in emergency more number of collections a day can be obtained. Frequent collections may result in a decline in concentration of sperm.

Dziuk *et al* (1954) collected good quality of semen from goats by electro-ejaculation method. This is however painful and injurious.

### Sexual behaviour and the act of copulation

The display of sexual behaviour by the buck is more elaborate than that of doe. It pays attention to the female, nose the perineum of the female,

followed by lip curling with head elevated. Bucks make low grunting and gurgling sound, licking the flank of the doe, nudging by extension and flexion of one fore leg, tilting the head, running the tongue in and out rapidly, pawing, rubbing along the side of the doe, attempting to push and guide the doe from other animals of the herd especially if the males are present. Sexual excitability and its intensity depends upon the sexual drive of the buck and external stimuli.

During copulation the buck mounts and makes frequent thrusting movements until the penis meets the vagina and then make the final deep thrust which is followed by ejaculation. Fielden and Barker (1964) reported that bucks have mated as many as 14 times a day. The number of services per hour is usually highest early in the morning and late in the evening. Beckett *et al* (1967) reported that the blood pressure in the corpus cavernosum of the penis at the time of erection in the buck was similar to that in the bull, about 1800 mm of Hg. In the buck warmth of the vagina is more important than pressure and friction as stimuli for ejaculation. The urethral process is engorged with blood. The urethral dilatation in the base of the process gets filled with semen and the semen is forced through the narrow orifice at the end of the urethra under considerable pressure producing a buck and forth spraying at the time of ejaculation.

### Consistency

The consistency varies from thin to thick depending upon concentration of spermatozoa.

### Volume

The ejaculate volume varies from 0.25 to 5 ml. but usually between 0.50 to 1.50 ml. Sharma *et al* (1957) observed an average volume of 0.8 ml. in Beetal goats. Patel and Raja (1973) reported the volume in Malbari bucks as  $0.5 \pm 0.03$  ml. Tiwari *et al* (1968) reported the mean ejaculate volume of Barbari and Sannen goats as  $0.657 \pm 0.03$  ml. and  $0.595 \pm 0.031$  ml. respectively. The differences between these breeds were significant when in a second trial the mean values obtained were  $0.52 \pm 0.06$  and  $0.997 \pm 0.055$  on once a day collection basis. Higher ejaculate volume may be associated with poor quality of semen.



Mass activity		Individual motility	
++++	Rapid cloud formation	+ 4	90 100% good forward motion
+++	Good cloud formation	+ 3.5	80 90% good forward motion
++	Strong flow with some thickening	+ 3	70 80% good forward motion
+	Poor flow	+ 2.5	60 70% good forward motion
-	No motility	+ 2	40 60% good forward motion or over 60% fairly good forward motion
		+ 1.5	20 40% good forward motion or 40 60% fairly good forward motion or more than 60% satisfactory forward motion
		+ 1	Less than 20% good forward motion or 20-40% fairly good forward motion or 40 60% satisfactory forward motion
		± 0.5	Little or no motion

Barbari bucks by Tiwari *et al* (1968) revealed an average sperm concentration of  $2000 \times 10^6$  sperm per ml of which 83% were counted as live

### pH

It varies from 6.2 to 7.0. Kurian and Raja (1965) reported the pH of semen of Malabari bucks as 6.3 to 6.7

### Live and Dead Count

Live and dead count is done by Eosin nigrosin staining. The standard for live sperm is 80-90%. The same stain can be used for determining the abnormal sperms.

The goat spermatozoa are highly susceptible for cold shock (Sahni 1967). Thus handling of semen in cold months should be done at 30°C.

### Morphological characteristics

The spermatozoa of buck have the same characteristics of that of the bull. Patel and Raja (1973) studied the characteristics of spermatozoa of Malabari bucks. They have reported the mean length, width and width at the base of the sperm head as 8.39, 4.37, 2.15 microns respectively. Length and width of middle piece were 11.81 microns and 1.06 microns. Length of tail was 41.26 microns. The motility

rate of ovine spermatozoa was reported to be 200 to 250 microns per second (Roberts, 1971). The total number of abnormal spermatozoa consisting of head, mid-piece and tail abnormalities should not exceed more than 10% for satisfactory fertility (Bretschneider, 1948; Dussardier and Szumowski, 1952; Blokhuis, 1957 and Maule, 1962).

### Biochemical characters

Fructose is the normal sugar providing a source of energy for spermatozoa in the semen of bucks as in the case of bull. It is produced in the seminal vesicles. The quantity of fructose was reported to be 250 mg/100 ml. of seminal plasma. The fructose level in the Malabari bucks was observed to vary from 320.00 mg. to 866.15 mg/100 ml (Patel and Raja, 1973). Citric acid in the buck semen was reported to be 40 mg/100 ml. (Roberts, 1971) and 647.15 mg/100 ml. (Patel and Raja, 1973). They have also reported that the levels of reducing substances in the semen are higher in the Malabari bucks than in the ram. Other substances are Potassium 90 mg/100 ml., Sodium 190 mg/100 ml., Phosphorus 375 mg/100 ml., Inositol 12 mg/100 ml., Sorbitol 92 mg/100 ml. and Glycerol Phosphorylcholine 1650 mg/100 ml. Goat semen contains a peculiar "egg" which is said

ing water at 18°C and then transferring it to a refrigerator at 4°C. In about three hours time the temperature gradually lowers to 5°C.

### Dilution

Test of the standard diluents at different rates have been carried out by different workers (Buhmann, 1952; Blokhuis, 1957, 1959). Glycine has not been considered as a good diluent (Roy, 1957). The addition of egg yolk has been considered useful for extending life of the spermatozoa. The egg yolk citrate diluent is suitable for storage of goat semen, the rate of yolk to diluent being 1:20. Blokhuis (1959) has reported that 1.50% sodium citrate, +5% of egg yolk is the diluent that gives satisfactory results.

Milk diluent has been found better than all other diluents (Sahni, 1967). John and Raja (1973) have reported that cow milk was superior for preserving goat semen. Milk should be boiled at 92°C for 10 minutes to render the milk free from spermicidal factor. The milk is then cooled to room temperature. It is then filtered to make it free from large fat globules. Antibiotics are then added.

### Rate of dilution

The semen is diluted to 1:4 and stored at 8°C. Hampel (1951) found that 60% of the spermatozoa showed good motility, 30% showed local motion and 10% were dead after 30 hours. The corresponding figures in semen stored at 4°C were 0.60 and 40% respectively. Tests of the standard diluents at different dilution rates from 1:1 to 1:15 have been carried out by several workers (Hampel, 1951; Buhmann, 1952; Blokhuis 1957, 1959). In the present state of our knowledge dilution rate should be kept at a level ranging from 1:1 to 1:5.

### Storage

The diluted semen can be stored at 8°C. Goat sperms are highly susceptible to cold shock and hence cooling from 30°C to 8°C must be done gradually within a period of 2-3 hours. When fresh semen stored at 10-12°C has to be transported, this is best done in thermosflask having the same internal temperature. Semen stored at 8°C can be transported in thermosflask containing a few lumps of ice, packed in rubber or plastic and wrapped in cotton wool.

### Insemination

The doe in oestrus is held and speculum lubricated with liquid paraffin is inserted and cervix located. Once the cervix is located, the inseminator will have no difficulty in inserting the pipette. In small goats the speculum should be inserted by careful turning. The pipette containing 0.1 ml. to 0.2 ml. semen is inserted through the speculum with the help of right hand and semen is blown on the mouth of cervix by slightly pressing the rubber bulb or by means

of a syringe. The pipette should not be penetrated deeply.

When the semen is rightly deposited in the mouth of the cervix, the inseminating dose for each insemination should be 0.1 or 0.2 ml. Freshly diluted semen with a minimum number of 20-50 million sperm per 0.1 ml. is required for obtaining the optimum conception rate. Tiwari (1967) reported conception rate of 70-80% with a 33 million sperm in an inseminating dose of 0.1 ml.

### Effect of seasonal variations on semen quality

In Netherlands, the mating season in goats is in the autumn and winter i.e. September to March, during which period the days are short and out-door temperature is low. If the temperature is below freezing point, it is observed that it has adverse effect on libido and the quality of semen. High temperature and atmospheric humidity have an adverse effect on semen quality and reaction time which shows individual variation (Mukherjee *et al*, 1953).

Sharma *et al* (1957) stated that the sperm concentration in goats is highest in winter and lowest in spring, the quality of semen per collection being largest in summer (3.00 ml.) compared to that in winter. They considered that best results are obtained when goats are attended to by the familiar persons. They also recommended the use of intersex goats as teasers.

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# Chapter 45

## Artificial Insemination in the Swine

The experimental work in artificial insemination in the Swine was first started in Russia by Milovanov in 1932. It's practical application was adopted in Japan by Niwa and co-workers in 1948. In Europe, the work on the Artificial Insemination in the Swine started much later. However a beginning was made in Hungary and the first piglets by this method were born in February 1955 (Pasztor, 1955 a, b). Thereafter, Polge (1956a) in England, Aamdal (1959), in Norway, DuMesnil Du Buisson (1956) in France further continued the experimental work.

The advantages of breeding swine by Artificial Insemination are similar to those in the bovine. This has particularly helped in controlling infectious diseases particularly *Brucella suis*.

Breeding Swine by the Artificial Insemination method has not spread extensively as in the bovine because of the species characteristics, the high cost of insemination, labour involved in insemination and difficulties encountered in deep freezing of boar semen.

### Sexual maturity

The age at sexual maturity in boars is same as that in sows. Fully developed spermatozoa are seen in the epididy-

mus at the age of 6-7 months and ejaculations can occur. However, even at 9 months of age, the fertility rate of boar is low. Sexual maturity is attained at one year of age. The quantity of semen at one year of age is half than that obtained from a four year old boar. In contrast to sows, high plane of nutrition influences early attainment of sexual maturity in the male.

The quantitative histological evaluation of the testicular tissue of boar revealed that the sperm output is around 12 to 17%, which is lowest of all the farm animals. Greater the frequency of collection of semen, poorer is the semen quality and fertility. This may be due to the shorter stay of spermatozoa in the epididymus. Even if the collections are made at longer intervals the sperm count is not high and thus the sperm output is not related to the longer period of sexual rest.

### Frequency of semen collections

Even if semen collections are made every day, the sperm count remains almost at the same level (20 millions/ml.) except for a little decrease in the beginning (Jondet *et al*, 1971).

During the first collection alone, majority of the spermatozoa are ejacu-

lated. This is probably on account of longer duration of coitus.

Repeated daily semen collections are not possible from the boar. Daily collection of semen leads to exhaustion of boar, deterioration in the semen quality and poorer libido (Schaez, 1963). The author has observed a decrease in volume and total count of spermatozoa when collections were made for three consecutive days. On the fourth day, the semen quality and keeping quality deteriorated to a great extent. In contrast to this, Aamdal (1964) observed the sperm count as 30, 43 and 45 millions/ml. on 2nd, 3rd and 4th day of collection respectively.

Schaez (1963) recommends 2-3 collections per week. He considers that young boars below two years of age should not be used more than twice a week. Reed (1969) and Becze *et al* (1976) recommend five days interval between two collections to maintain the libido and sperm concentration until the age of 4-4½ years. Jondet (1971) recommends only one collection per week. With this procedure he observed the duration of ejaculation as 5.82 minutes, volume of ejaculate as 296.9 ml. and total sperm count as 95.4 million/ml.

### Libido

Libido of boars varies with the breed. It is stronger in the primitive breeds than that in the Landrace. Libido and fertility both are greatly influenced by the nutritional status and management practices.

In young boars, the libido is not strong but it improves as the age advances. If reaction time exceeds 45 minutes, it is not desirable to use such boar for breeding (Schaez, 1963). Free movements in the paddocks keep

the boars in trim condition and help them to maintain their normal libido. Boars becoming heavy and sluggish at early age is the frequent cause of culling. Any febrile condition reduces the libido and fertility and it may take 4 to 6 weeks for the boar to return to normal fertility.

Decline in libido and fertility is noticed in boars after the age of 5 years. However Aamdal (1964) has noted high fertility in certain boars beyond 8 years of age.

### Collection of Semen

On account of long reaction time in boars resulting in restlessness of the sows, collection of semen on dummy teasers has become the practice. Dermo-plastic dummies as designed by Pasztor (1955) look more natural but being costly simple dummies covered with saccloth or leather are more frequently used. In the earlier dummies, artificial vagina used to be fixed inside and a window at the right side of the dummy facilitated collection of semen. Boars can be easily trained to mount the dummies since the immobilisation induces stimulus which provokes the ejaculatory reflex. Young boars with strong libido can get accustomed to the dummy after few mounts. For the training of boars reared in large stud farms, Becze *et al* (1973) have designed a model in which a oestrus sow can be placed in. After the training, the sow in heat is withdrawn.

### Artificial Vagina

Artificial vagina for boars was designed by McKenzie (1931). In the Aamdal type artificial vagina, a spiral shaped metal armature was fixed attached to the end of vagina. Foam rubber piece fixed

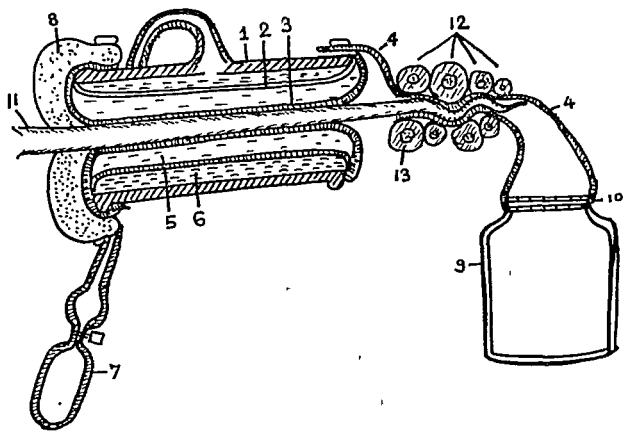


Fig 154 Artificial Vagina for Boar (Cross-section)

- 1 Outer wall of the vagina
- 2 Inner air tube.
- 3 Inner water tube
- 4 Funnel
- 5 Water
- 6 Air
- 7 Air pump

- 8 Foam rubber piece.
9. Plastic bottle.
- 10 Double perforated lid
- 11 Penis of boar
- 12 Fingers of right hand.
- 13 Thumb.

at the entrance of the artificial vagina has helped in obtaining germ free ejaculates, avoiding the preputial contents (Fig 154) Artificial vagina heated electrically to maintain a constant temperature is now available, otherwise difference in A.V. temperature will distract boars from their reaction to service. So far there is no ideal model of A.V. in which fractionated sections of the semen can be conveniently collected.

Niwa (1959) and MacPherson *et al* (1960) found it exceptionally convenient to collect semen from boar by hand stimulation compared to the usual method of collection by A.V. The hand method of semen collection is as follows: The

operator stands on the right side of the dummy. After the boar has mounted he catches the twisted glans penis by gloved hand and provokes ejaculation by exerting slight pressure on the penis which simulates rhythmical compression of the cervix. The semen is collected in pyrex bottles provided with gauze filters. This method prevents contamination of semen in fractions. This method also helps in avoiding interruptions of the reflexes (Fig. 155).

Sexual behaviour of boars during natural service is as follows: The pre service behaviour of the boar is very characteristic. First the boar turns agains

the sow, then it comes into close proximity lifting and prodding the sow and at the end besides increase in sexual excitement, the boar then moves towards the genitalia. At the same time the sow exhibits reflex of immobilization like a "Saw-Horse" attitude, pushing its tail to one side, tilting its head and trembling ears can be noticed. Penile movements are observed prior to mounting. With few attempts the boar mounts with erectile penis. It takes anything from 1 to 20 minutes for the boar to get ready either for natural service or for collection of semen in A.V. At the height of excitement the boar having mounted clasps the back of the sow with its free-legs, bringing its hind limbs closer to the sow and makes searching movements. After 1 to 2 minutes friction movement associated with bending of the back of the sow, the penis penetrates into the cervix and it gets locked up in the longitudinal folds of the thickened walls of the cervix. In the sow, during dioestrus, the cervix is in relaxed condition whereas, during oestrus it becomes thick, oedematous and gets locked, which is in contrast to the cervix of the cow. The locked up condition leads to penile rhythmical contractions, the strength of which is directly proportional to the degree of sexual excitement which is observed externally (Smidt *et al*, 1969). The pressure of the cervix causes the ejaculatory reflex. At times young boars leave the sow after some friction movements and then after a short period they begin to copulate again. Ejaculation extends over 5 to 25 minutes or even more. On completion of the ejaculatory act, the boars get exhausted in consequence of which dismounting is delayed. Few boars get ready for the second ejaculation.

### Ejaculation

The ejaculation of boar is in fractions. The first fraction which is watery and discoloured is the secretion of the urethral glands. The quantity is about 10 ml. and is sperm free. The deflection of this secretion is over at the height of excitement. Its importance is in rinsing the urethral tract which usually has a high bacterial count. It is not advisable to mix this fraction with semen. During the subsequent phase of ejaculations the boar is usually quiet. The volume of second fraction is about 40-80 ml., is dense and milky and contains about 80% of spermatozoa.

The third fraction consists of 150-200 ml. of thin fluid with low sperm concentration. This fluid originates from the secretions of seminal vesicles and prostate gland which is known as seminal plasma.

The fourth fraction is gelatinous and secreted by bulbourethral glands.

Ejaculation is not always continuous and it takes place in five separate waves. Its duration varies from 15-20 minutes. In boars with high vigour, the ejaculatory response is quicker. It is also influenced by intensity of oestrus in sow and pressure of cervix on boar's penis. In fractionated semen collection, pre-sperm fraction is not used, whereas the 2nd and 3rd fractions are used for insemination. During collection, the gelatinous substance is held up by the filter.

### EVALUATION OF SEMEN

The average volume of semen varies from 50-500 ml. Besides this occasional, seasonal and individual variations are also observed. Under continental conditions the volume of ejaculate is lar-



gest during summer and smallest in winter. It also depends upon method and frequency of semen collection. Jondet *et al* (1971) recorded 30% higher volume and 10% higher total sperm count in the hand method of collection as compared to collection by artificial vagina.

The first two fractions are homogeneous fluids, the colour depending on density can be bonewhite, milky and bluish or watery. The semen has a characteristic odour. Yellowish, greenish or blood tinged semen is abnormal. Presence of mucus noticed on the collection bottle is also considered abnormal. Urine or other unpleasant odour indicates contamination with extraneous material.

The pH of semen ranges between 7-8. The pH becomes more alkaline with the diseases of accessory glands.

Normal ejaculate contains a few epithelial casts. Presence of leucocytes indicates an inflammatory process. The semen usually contains a few bacteria. With the older types of artificial vagina, contamination with preputial diverticulum occurs frequently. Aamdal (1961) has successfully shown that the contamination could be reduced by removal of preputial diverticulum. The contamination can be minimised by using newer types of Artificial Vagina and hand collection.

Seminal plasma of boar is characterised by high calcium content. Besides fructose and citric acid, it contains meso-inositol and ergothionine.

There is little variation in density of semen due to breeds but it is affected by the method of collection viz. whether by hand or artificial vagina. The boar semen is not so dense as that of the bull. The density can be determined by photoelectric colorimeters.

The total sperm count varies from 100,000 to 500,000 cmm. The total count varies from 15,000 to 50,000 cmm. when collected daily and from 2,65,000 to 5,51,000 cmm. when collected at weekly intervals. The total sperm output in a single ejaculate may sometimes be 10 billions.

Mass activity can be observed under microscope where the sperm motion is very vigorous. Ejaculates having spermatozoa with crosswise, circular or oscillating movements or those with no motion at all cannot be used for insemination.

Live and dead sperm count can be done by vital staining technique. Ejaculate with more than 80% active spermatozoa is considered to be 'very good' whereas the one with 70% is considered as 'good' and the one with 60% as 'satisfactory'. Ejaculates with less than 70% active spermatozoa should not be used for insemination. Paredis and Vandeplasse (1961) reported significantly higher conception rates where semen containing more than 75% active spermatozoa were used.

The spermatozoa of boar remain in anabiotic state at room temperature after 6 hours, sooner at low temperature and immediately after collection. Simultaneous warming and shaking resuscitates the sperm action. This property is made use of while storing semen at higher temperatures.

The morphological abnormalities can well be differentiated when stained with Alanin or Giemsa stains. The boar semen has an abnormal count to the extent of 14.2% (Passtor, 1955 a, b). It is not advisable to use semen for insemination, if the total sperm abnormalities exceed 25%.

In boars, morphological abnormalities are of frequent occurrence compared to other species. Amongst these, the distal protoplasmic droplets are more common. Such spermatozoa are visually non-motile. In a normal ejaculate, incidence of spermatozoa having protoplasmic droplets is rare. Increase in frequency of collection, raises the incidence of proto-drops. If young boars exhibit 50-60% of such abnormalities conception rate will be reduced to 20-30% resulting into small litter size. Such boars in due course of time become sterile and as such it is recommended to cull them as early as possible (Becze and Szilvassy, 1972). As the keeping quality of boar semen varies to a great extent, it is desirable to evaluate the diluted semen from time to time. The number of motile spermatozoa varies with individual and ejaculates during storage and at times the motile spermatozoa may decline by 50% after storage of 6-8 hours.

### Dilution and storage of semen

Addition of diluents containing protective colloids and sugar will help the boar spermatozoa to survive. Storage of boar semen at 15° to 20°C has been universally accepted. Among colloids serum of different species has given good results. Egg yolk and pasteurised skim milk is favourably used to prolong the life span of the spermatozoa significantly. The other commonly used diluents contain 25 to 30% egg yolk to which sodium citrate or bicarbonate and glucose are added. As large quantities of foreign protein in egg yolk cause local or general immune reactions, the use of egg yolk has become limited. On the other hand diluents containing organic material favour multiplication of bacteria in the semen stored at room temperature. Diluents containing inorganic substances

are therefore advocated. It is known that sugar and other organic substances increase metabolism while as carbon dioxide suppresses the spermatozoan activity and thus help in preserving their nutrients. Diluents containing carbon dioxide have a favourable effect on the survival of spermatozoa and hence are used on large scale for dilution of boar semen. This involves laboratory facilities and as such it is not possible to adopt this method in field conditions. Besides, evaluation of diluted semen becomes difficult. In order to avoid bacterial contamination during storage addition of antibiotics such as penicillin, streptomycin and polymyxin is recommended. It is generally believed that neat semen can be stored better than the diluted one. It is recommended that neat semen should be diluted as and when required for insemination. It is desirable to have the neat semen and diluent warmed to body temperature. In case of prediluted semen it may be further extended prior to insemination. Semen sample should be diluted gradually.

The dilution rate depends upon the total sperm count in the ejaculate and the total spermatozoa in a single dose. The usual dilution rate for boar semen is 1:3 to 5. The maximum dilution rate should not exceed 1:10. Presence of air in the storage vial or slight agitation during transport have no harmful effect on the spermatozoa.

The following diluters are commonly used for extending boar semen.

1. Sodium citrate	20 gm
Sodium bicarbonate	2 gm
Potassium chloride	0.1 gm
Glucose-D	30 gm
Sulphanilamide	10 gm
Penicillin	1 million I.U.
Streptomycin	10 gm
Glass distilled water	100 ml
pH value 6.0 to 8.2 at +35°C	

It is necessary to pass carbon dioxide for 10 minutes until the pH value reaches 6.4 to 6.5. The temperature of the semen can be maintained in the refrigerator by cooled crystalline acetic acid whose melting point is  $+15^{\circ}\text{C}$ . Prior to evaluation of the semen the sample has to be warmed to  $+25^{\circ}\text{C}$  and  $\text{CO}_2$  is removed by shaking machine. Semen stored by this method can be used for 2-3 days.

## 2 Diluent of Plischke (Triplex) —

Glucose D	120.0 gm
Ethylenediamine acetate	7.4 gm
NaOH (Basic solution 1%)	16.0 ml
Sodium citrate 35.5%	20.0 ml
Penicillin	100 000 i.u.
Streptomycin	0.5 gm
Glass distilled water	2000 ml

## 3 Other Improved diluters of Plischke —

Glucose D	40.0 gm
Sodium bicarbonate	1.0 gm
Sodium citrate	3.8 gm
Cheloplex Helaton	2.6 gm
Egg yolk	100.0 ml
Combiotic	2.5 ml
Glass distilled water	1000 ml

The above diluters can preserve boar semen for 3 to 6 days at a temperature of  $+10^{\circ}\text{C}$  to  $+15^{\circ}\text{C}$ .

## 1 Further improved diluent of Plischke —

Glucose D	120.0 gm
Solution i.e. EDTA	7.4 gm
Sodium bicarbonate	2.4 gm
Sodium citrate	7.5 gm
Penicillin	100 000 i.u.
Streptomycin	1.0 gm
Glass distilled water	2000 ml

The chemical composition of boar semen as cited by Mann (1961) is presented in Table 61.

## Detection of Oestrus in Sows

### USE OF TEASERS

Use of teaser is of great importance in detecting the oestrus and to determine the time of insemination. Sow in oestrus plays a predominant part in attracting the boar. However immobilisation of the sow in heat has also a stimulating effect on the boar. Not more than 20 to 30 sows should be left for detection of heat to one teaser. It will thus be possible for the teaser to reach every sow. Using this method 10 to 15 minutes are enough to detect all the sows in heat in a group. Vasectomy of the boar is not necessary because there is enough time to separate the boar from the sows in heat.

### RIDING TEST

This test can be done by the farmer or breeder. The immobilisation reflex of the sow can be evoked either by exerting pressure on the sacrolumbar region or by sitting in a riding position on the hind part of the sow. Without the teaser man can evoke this reflex in 90% of sows (Signoret, 1970). Proximity of boar increases the efficiency of the test. Immobilisation reflex can be easily provoked if the sow smells the boar odour from the clothes of operator. Fairly good results have been claimed by use of different acrosomes containing boar pheromones which help in accelerating the reflex of sow but not the increase in conception rate. Favourable results have been obtained by simulating the grunt of boar. Generally one minute is more than enough for the skilled operator to evoke this reflex. However some sows may not manifest heat reflex in presence of the operator others stop easily whereas some become immobile only in the presence of the boar.

Table 61

## BOAR SEMEN: MAIN COMPONENTS

Results are average values (range in brackets) expressed in mg/100 ml., except for CO<sub>2</sub> content (ml/100 ml). Data on dry weight electrolytes and total nitrogen.

Dry weight	4600	(2,900-6,200)
Chloride (cl)	328	(258-428)
Sodium	646	(280-837)
Potassium	243	(83-382)
Calcium	5	(2-6)
Magnesium	11	(5-14)
Inorganic phosphorus	17	—
CO <sub>2</sub>	50	—
Total nitrogen	613	(334-765)
Non-protein nitrogen	22	(15-31)
Urea	5	—
Uric acid	3	—
Ammonia	1.5	(0.5-2)
Fructose	12.6	(2.5-18.5)
Lactic acid	27	—
Citric acid	129	(36-325)
Total phosphorus	357	—
Acid soluble phosphorus	171	—
Lipid phosphorus	6	—
Ergothioneine	15.2	(5.7-29.5)
Ascorbic acid	3.5	(2-5)
Inositol	532.0	(382-625)
Glycerylphosphorylcholine	171.0	—
Sulphur	16	(12-22)
Creatine + creatinine	8.5	(4-11)

## Time of Insemination

Vagina of sow is bowl shaped cavity that becomes narrower towards cervix. A simple rubber or plastic tubing cannot be introduced easily into the cervix on account of its longitudinal folds and also the back flow of semen cannot be prevented. Insemination catheter devised by Aamdal (1961) is provided with plastic cup which can be inflated after its in-

troduction. The cuff on inflation becomes adherent to the cervix thereby preventing the back flow of semen. (Fig. 156). The catheter designed by Melrose and O'Hagan (1961) was made of rubber plastic and simulated boar penis. This can be smoothly introduced by twisting it to the right which locks the cervix and prevents backflow of semen. In the sow the site of deposition

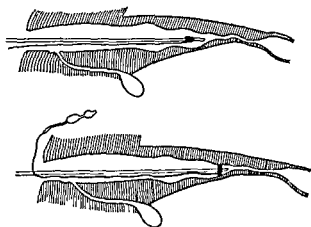


Fig 156 AI in the sow

- (a) Inseminating catheter pushed far into the cervical canal  
 (b) Plastic cuff at the tip of the catheter has been inflated

of semen is in the uterus proper. This is very important for obtaining high rate of fruitful conceptions as compared to a very low rate when the semen is deposited in vagina or cervix. Spiral catheter designed by Melrose and O'Hagan (1961) gave 3% higher conception rates compared to inseminations carried out by catheters with cuff (Jondet *et al*, 1971).

**Introduction of semen into the uterus** is carried out by slight pressure. Large sized syringes are not convenient for this purpose. Conical plastic bags attached directly to catheter are very practical and semen can be evacuated by manual pressure.

The sow is secured in a sternal recumbent position or a narrow box or by a person sitting on the back during insemination. Frequently a slight pressure on back is enough to evoke immobilisation during insemination. The vulval lips should be cleaned with sterilised cotton before introduction of catheter. It is necessary to ascertain that the oestral discharge is normal.

Spiral tipped catheter can be easily passed through the cervix and if the catheter does not slide back semen can be syringed with slight pressure preferably by gravitation (Fig 157). Forceful introduction would provoke uterine contractions resulting in back flow of semen. Insemination usually takes 5-10 minutes. In case there is back flow of semen during insemination, the catheter has to be refixed properly in the os uterum.

The quantity of semen plays a great part in transportation of spermatozoa and hence large volume of semen is required. The recommended insemination dose varies from 100-250 ml in adults and 100 ml in gilts. However, fairly good results have been obtained by using even 50 ml of semen (Jondet *et al*, 1971).

Table 62

SPERMATOZOA REQUIRED PER INSEMINATION DOSE

Author	Year	Billion
Polge	(1956, b)	5
Aamdal	(1959)	1
Paredis and Vandeplasseche	(1961)	7
Bennet and O'Hagan	(1964)	2

However according to Aamdal *et al* (1958) no fixed rule can be laid down since it is influenced by various factors such as quality of diluents, age of sows, site of deposition, quality of semen and duration of insemination. Jondet *et al* (1971) considered that with fresh semen sperm count per dose can be decreased below 25 billion without affecting the conception rate adversely. The conception rate with preserved semen can be increased from 15 to 61% by increasing

the sperm count from 4 to 12 billion spermatozoa per dose. Bennet and O'Hagan (1964) achieved good results with 2 billion spermatozoa in semen preserved for 5 days. Further, they observed significant decrease in conception rate and litter size, when the sperm count was reduced below 1 or 0.5 billion.

### Time of Insemination

The correct time of insemination greatly influences conception rate and litter size (Aamdal, 1964 Bennet and O'Hagen, 1964 and Smidt and Ellendorf, 1969). Ova remain viable for about 10 hours after ovulation. Fertilizing ability of the spermatozoa however lasts for 25-30 hours (Roberts, 1971) and longest recorded is for 50 hours (Jondet *et al*, 1971). After natural service the ova are usually surrounded by large number of sperms compared to insemination. Less spermatozoa are found in the oviduct using intra-vaginal or intra-cervical insemination than in the case of intra-uterine insemination (Hancock, 1963). Incorrect timing of insemination results in small litter size due to degenerative changes occurring in the gametes. As such insemination should be done prior to ovulation. The onset and duration of oestrus varies in sows. The only criteria known so far, for the correct timing of insemination is the onset of oestrus. The time and onset of oestrus depends to a great extent on the intervals between detections. Many experiments have been carried out to find out the time of ovulation viz. vaginal cytology, vaginal temperature and pH of vaginal mucus. These are not reliable as is found in case of pH that there is variation in values of electrical resistance from cycle to cycle. It is therefore very difficult to decide about the exact time of ovulation.

Oestrus in the sow can be divided into three phases. The true oestrus in sows is one when the sow exhibits reflex of immobilization. The sexual excitement though appears at least a few hours earlier it develops gradually and disappears after several days.

Arrangement to feed them *ad lib* should be made by fixing self feeders, so that the time of feeding will not disturb the sexual behaviour. Detection of heat is carried at 3 hours interval. Becze observed the immobilization reflex, occurring at a relatively short time (within 3 hours) in between the checks. The reflex was not gradual. Thus he has shown that the onset of oestrus in sows can be determined by frequent and intensive checks.

Concerning the manifestation of oestrus Becze *et al* (1973) observed two peaks within a day. First one was in the morning from 4 hrs. to 10 hrs and another one in the evening from 19 hrs. to 22 hrs. Besides these two periods cited above, they have hardly observed oestrus during other periods of the day and night. They have further observed by autopsy that the earliest time of onset of ovulation was 36 hrs. after the possible immobilization reflex was evoked by the sow and the series of ovulations in sows were completed by about 40 hrs.

Based on the above experiment Becze and Szilvassy (1972) consider that checking of sows by 9.00 hours for detection of heat in sows which have begun cycling previous night and early morning hours is necessary. By this time the sows expressing oestrus during night hours have passed 10-14 hours, whereas 1-5 hours in those which have expressed heat very early in the morning. Thus when inseminations are carried out in the

afternoon the sows are already in 17-21 hrs. and 8-12 hours of heat respectively. They are in 32-36 hrs. and 23-28 hrs. of heat when second inseminations are carried out next day morning. For large commercial herds, detection of heat at 9.00 hrs. and insemination during afternoon hours followed by repeat insemination next day morning is found to give better results.

### Conception rate

According to Madden (1959, 1960) conception rate at first service was 68 to 86 per cent in natural mating. Melrose and O'Hagan (1961) reported a conception rate of 80%. According to Aamdal (1961) conception rate was 75% and the litter size 9-11.

In A.I. method conception rate is lower and litter size is generally smaller compared to that of natural mating. Jondet *et al* (1971) reported 55-60% conception rate by A.I. method but deviations are significant. In gilts conception rate is usually low i.e. about 50%.

Best results 75% are usually obtained in sows found in heat within 9 days following weaning. Litter size varies with the breed. In Hungary, the average litter size was 9.6 (National average of conception rate with 1st insemination was 55-60% with a range of 40-75%). This difference can be attributed to variation in feeding, housing and management systems (Becze *et al*, 1976).

With improved technology in detection and insemination, higher conception rates can be achieved as well as this lessens the burden of maintaining large number of boars required for double service to each sow. Variation in conception rate is due to the fact that the data

regarding conception to 1st service and actual farrowing is not accurately maintained. The evaluation of conception rate based on non-return % as used in cattle cannot be applied in sows—since 10-15% sows may not exhibit heat even though they have not conceived. In sows conception rate can only be estimated by farrowing and litter size. Early confirmation of pregnancy in sows is of great economical value. Pregnancy diagnosis at a very early stage can be carried out by use of ultrasonic instruments based on the principle of Doppler echography and this can be done between 28-35 days of pregnancy (Szilvassy *et al*, 1976).

In Europe, collection of semen and insemination is done on the same farm in large commercial units, which is economical. In small farms insemination service is rendered on the farm proper but in some countries arrangements are even made to despatch semen by post along with necessary equipment and instructions for inseminations.

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# Chapter 46

## Artificial Insemination in the Dog

An Italian Scientist, A. Spallanzani was the first to place on record the successful results of artificial insemination in the bitch in the year 1780.

Heape (1897), Hermansson (1935), Hancock and Rowlands (1949), Hadi and Sane (1968) and Deshpande *et al* (1970) reported successful results by artificial insemination in the bitches.

In most of the countries, this method is being used supplementary to natural mating. In India, Deshpande *et al* (1970) have inseminated number of bitches offered for insemination for reasons that mating otherwise is impossible. Encouraging results have been obtained.

### Advantages and Limitations

It is considered that there are four main advantages of this method of breeding by Artificial Insemination over natural mating in dogs.

1. To bring about effective service in good time which for some physiological or psychological reasons, is either difficult or impossible.

2. When natural mating is impossible due to geographical distances, artificial insemination is the only solu-

tion to use superior type of dog with desirable traits.

3. To extend the usefulness of approved stud dogs.

4. Control of coital infections.

### Limitations

Certain countries like United Kingdom have no objection to carry out insemination within the country but the Ministry prohibits import of canine semen from countries that are not free from rabies. Permission from Kennel club is necessary prior to artificial insemination in dogs, otherwise the progeny from such a conception is disqualified.

### Special features of the reproductive system in the dog

The seminal vesicles and Cowper's glands are not present in the dog. Prostate is the only accessory sex gland. This is located at the neck of the bladder and it completely surrounds the prostatic portion of the urethra where it is adherent. The prostate gland empties into urethra through many small openings in the urethral wall on either side of the urethral crest. In older dogs, hypertrophy of this gland is commonly observed.

The testicles, epididymis and ductus deferens are similar to those of the bull, the root of the penis is attached to the ischium. The penis has a body and a relatively large glans penis with a bulb at its caudal end. The anterior portion of the body and most of the portion of the glans penis has a bone, os-penis. The os-penis has a deep ventral groove for the passage of the urethra.

In the dog, the mechanism of erection is not very well understood. The bulb of the glans penis enlarges during erection and serves as the male part of the locking device, which results in a tie during coitus.

### Special features in the bitch

The reproductive organs of the bitch consist of the vulva, vestibule, vagina, bicornuate uterus and ovaries. The vulva looks relatively small during anoestrus condition but during oestrus, it becomes turgid and tumefied. As the time for mating approaches, the texture of the vulva changes from rigid to soft and spongy. The vestibule extends from vulva to the urethral opening. This part of the canal is vertical in direction, except during copulation when it assumes almost horizontal position.

The reproductive cycle in the bitch, which is completed twice each year, extends from 170 days to 200 days. Anoestrus period, seldom lasts for more than 2 months. A vaginal smear made during anoestrus period is primarily composed of leucocytes and epithelial cells. In the bitch, the prooestrus period is the time which extends from the first appearance of a bloody discharge to the time of first acceptance of the male. The period varies from 7 to 9 days. Vaginal smears made during this period will

show erythrocytes and cornified epithelial cells. Leucocytes are markedly absent. Oestrus in the bitch is the period from the first to the last acceptance of the male. The period may extend from 5 to 10 days with an average of about 9 days. Ovulation usually occurs at about the second day of true oestrus (Doak *et al*, 1967). The vaginal smear during this period will show few erythrocytes and more disintegrating cornified epithelial cells. At the end of oestrous period the bacterial count is high.

A vaginal smear made during metoestrus period will show the appearance of leucocytes and the gradual change of the cornified epithelial cells to those having the characteristics of anoestrus period.

### Act of copulation

A dog is usually attracted to the bitch in oestrus much earlier before the bitch is ready for the mating. The acceptance period is only during true oestrus. Some bitches will enjoy courtship or tease a stud dog for a full day before they get ready for service. In case the stud dog is not vigorous, the teasing period is likely to be extended. A good stud dog may not allow any teasing but will mount a bitch immediately on contact.

In true oestrus when the bitch is ready for mating, it turns her genitals towards the stud and swing the tail to one side. The vulval opening enlarges in such a manner that the vestibule becomes horizontal in position. The bitch stands quietly for the male to mount. On mounting, the dog protrudes the anterior portion of the glans and attempts to contact the vulva. On successful contact, the penis is thrust into vestibule, the prepuce being pushed

back of the bulb of the glans and the bulb thereafter is forced in the vulva. It is peculiar to find in the dog a series of short thrust movements and during this part of copulatory act, a true erection takes place. Due to such frequent copulatory movements, the entire glans penis including the bulb becomes greatly engorged and enlarged. The vulval lips tightly grasp the penis just posterior to the bulb which results in a tie, between the male and the female.

With the coital thrust, ejaculation begins. During the first phase, there is an emission of a clear watery fluid which continues to occur until the erection is complete. At this stage, the thrust movements cease but the ejaculation continues into the second phase. During second phase the ejaculate is whitish milky fluid with large number of spermatozoa. Pulsations at the root of the penis can be felt during ejaculatory action. This phase extends from 5 to 15 minutes. During this phase, frequently the dog turns over the female, stands back to back with a firm tie which may last for 15 to 30 minutes.

#### Collection of semen

It is usually experienced that whenever a bitch in oestrus is used as teaser the response to artificial vagina is fairly good as compared to those in anoestrous condition.

The artificial vagina developed by Harrop (1954) consists of a cylindrical bladder between the line and the lumen of the artificial vagina which provides the space for water and a hand pump is provided to impart pulsation to the liner. This device provides necessary sensory stimulus to the dog and the need for a teaser bitch can thus be eliminated. It is possible by this device to obtain complete ejaculate. It is more

hygienic and is preferable to digital manipulation whereby laceration is likely to occur. Collection of semen by electrical stimulation is inadvisable (Christensen and Dougherty, 1955).

#### Technique of collection

The collection technique followed by Deshpande *et al* (1970) is as follows:

In certain dogs before collection of semen sufficient time was given for the courtship. In all cases the semen was collected by massage method. A gloved hand was lubricated by sterile liquid paraffin. To begin with gentle massage was done on the skin covering the penis and the surrounding region of the abdomen. When erection occurred within a minute or so gentle massage on the skin of protruded penis was done by the lubricated hand. Pressure was exerted on the root of the penis in the region of ischio cavernosus muscle and on the bulb. With effective stimulation a pulsation was felt within about 5 to 10 minutes. This was indicative of commencement of the ejaculation. A wide mouthed sterile glass tube was held close to the tip of the penis. At this time massage was discontinued and intermittent pulsating pressure was exerted on the bulb of the penis. Care was taken not to introduce the penis in the glass receptacle for fear of development of inhibitory reflex due to contact with cold glass and of contamination. Lubrication applied was just sufficient in order to avoid its presence in the ejaculate proper. In case measuring test tube is used for collection of semen there is every danger of penis getting tube locked due to thrust reflex resulting injury to penis. If massage has to be done for a longer time it may cause bleeding due to friction.

### Artificial Vagina

The Artificial vagina designed by Harrop is preferred. It is prepared by pouring hot water at 45°C so that when it is ready, the temperature inside the liner is about 40°C which is suitable to commence collection. The lumen of the liner should be adjusted by means of the pump to the size of the dog's penis. Lubrication to the inside of the liner is not necessary as with other animals, since in true oestrus the vagina of the bitch is normally dry. (Fig. 158). To begin with, the penis is grasped behind the glans and massaged very gently. Within a short time the penis will show erection and it is then directed into the artificial vagina. The operator should hold the artificial vagina firmly in proper position as the dog will immediately begin thrust movements. By the other hand the operator should try to

retain the grasp on the penis behind the bulb. An assistant may be asked in the mean time to pump gently so that the penis comes in direct contact with the artificial vagina from all sides and also to create pulsation which is necessary to stimulate the dog for complete ejaculation.

In the dog the ejaculate occurs in three separate fractions. The first fraction is a clear watery fluid with no spermatozoa and may be from the urethral mucosa — the glands of Littre. The second fraction is mostly white in colour more viscous in consistency and this is the main sperm bearing fraction. The third fraction consists of prostatic secretion which is clear in appearance, watery in consistency and contains no spermatozoa (Freiberg, 1935).

According to the size and breed of the dog, the volume of each of these fractions vary to a great extent. Variations have also been observed between dogs of a similar breed and the same animal on different occasions. The average volume and rates of ejaculation are as follows:

Volume (Boucher <i>et al</i> 1958)	Rate of ejaculation (Harrop, 1955; Boucher <i>et al.</i> 1958)
First fraction 0.25 — 2 ml.	30-50 seconds
Second fraction 0.5—1 ml.	50-80 seconds
Third fraction 3 — 25 ml	3-30 minutes.

Alifanov (1935) reported that spermiogenesis is a continuous process and normal amounts of mature spermatozoa were restored from 24 to 72 hours. Similar observations have been recorded by

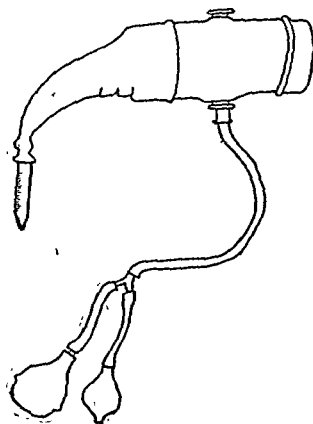


Fig. 158. Canine Artificial Vaginal (Harrop model).

Boucher *et al* (1958) and Harrop (1955). However they observed that frequent collections less than every two days, resulted in the decreased number of spermatozoa per collection.

It is not certain if every dog will react to spontaneous collection. They require to settle down and get used to the surrounding, operator and the method.

### Semen evaluation

#### Volume

In the dog ejaculates vary greatly in volume and sperm density due to great fluctuation in prostatic secretions. The volume depending on the breed of dog; ejaculate varies from 2 to 19 ml.

#### Colour

The colour of the dog semen may vary from light grey to milky white. The opacity varies directly with the density. After collection the semen gradually separates in two layers. The spermatozoa settle at the bottom. Deshpande *et al*

(1970) on 30 dogs of 9 breeds, observed the seminal attributes as under (Table 63).

The dog semen is thin and it is quite likely to happen, that the sperm bearing fraction does not get evenly mixed with the whole ejaculate. On many occasions when semen is drawn for examination from various points in the ejaculate volume, it may give deceptive picture if the sample is drawn from the thinner portion. It is therefore advisable to examine semen from different portions before drawing final conclusions with regard to mass activity and motility.

#### Density

The consistency of dog semen may vary from watery to viscous. The spermatozoal concentration per unit volume is carried out in the usual manner by use of the haemocytometer by diluting samples to 1:20 (Boucher *et al*, 1958) or 1:200 in formol saline (Harrop, 1955).

Table 63  
EVALUATION OF DOG SEMEN

Sr. No.	Breed	No. of observations	Volume in ml. Average (range)	Colour & consistency	Mass activity. (grade + to +++)	Motility of spermatozoa grade 1 to 5 average / range
1.	Pomeranian	8	2.06 (0.5-3.0)	Thin cloudy.	+++	2.87 (2.0-4.0)
2.	Pekingese	8	1.87 (1.5-2.5)	"	++(+)	2.43 (1.0-3.0)
3.	Toy Poodle	2	1.35 (1.2-1.5)	"	++(+)	2.75 (2.5-3.0)
4.	Dachshund	1	1.0	"	++	2.00
5.	Shetland sheep dog	6	1.0 (2.0-8.0)	"	++(+)	2.75 (2.0-4.0)
6.	Golden Retriever	1	5.0	"	++++	4.0
7.	Afgan Hound	2	1.00	"	++(+)	2.00
8.	Cocker spaniel	1	1.25	"	++(+)	2.50
9.	Alsatian	1	5.9	Thin milky	+++(+)	3.5

Table 64  
SPERM CONCENTRATION IN THE DOG (MILLIONS/ML)

Range	Average	Author
14—189	—	Alifanov (1935)
10— 90	—	Lambert & McKenzie (1940)
6— 64	27.5	Hancock and Rowlands (1949)
88—588	—	Nooder (1950)
4—540	125	Harrop (1955)

Various workers have reported the range of sperm concentration in million per ml. as above (Table 64).

The density is subject to variation depending on the method of collection whether it is a fraction or whole ejaculate. Digital manipulation gives higher concentration as compared to collections made by artificial vagina where the quantity of the total ejaculate obtained is usually greater.

semen individual movements of the spermatozoa are very brisk and are best studied under coverslip.

#### Hydrogen-ion concentration

Harrop (1955) observed that immediately after collection the average pH of normal dog semen is 6.75 (5.8-6.9). The pH of the individual fraction observed in his studies is as follows. 1st fraction 6.37; Second fraction 6.10 and Third fraction 7.20. Boucher *et al* (1958) reported that the pH of the dog semen varied according to the method of collection; (1) collection in artificial vagina without a teaser bitch 6.59, (2) digital manipulation without a teaser 6.52, (3) digital manipulation with a teaser 6.72. Deshpande *et al* (1970) observed in Alsation dogs, the fractionwise variation in the seminal attributes as follows (Table 65).

Table 65  
FRACTION-WISE SEMINAL ATTRIBUTES IN THE DOG

	Reaction time in seconds	Volume in ml.	Colour & consistency	Motility of spermatozoa	pH of semen	Sperm concentration	% of Dead sperm	% of abnormal sperm
First Fraction	30	3.2	Cloudy Thin	+ 3	6.6	—	—	—
Second Fraction	—	1.3	Milky Thin	+3.5	6.0	480	1	2.8
Third Fraction	—	1.4	Watery Thin	0.0	6.2	—	—	—

## Sperm morphology

Indian ink eosin nigrosin or Case rectt's staining solution are popularly used for staining semen slides. Normal semen in the dog should not contain more than 20% abnormal spermatozoa.

## Chemical composition of semen

In the dog the prostatic secretion is watery and colourless with the presence of strong proteolytic enzymes as well as some phosphatases and glucuronidases. A detailed study of biochemical characteristics of dog semen is given in Table 66 (Mann 1964).

It is interesting to note that in dog semen there is a high content of copper and zinc. Semen samples analysed by Birtlett (1962) contain seven times as much copper and twenty times as much zinc as corresponding blood samples in the dog. King and Mann (1959) reported that in the dog semen sample there is practically no fructose, citric acid or sorbitol. This proves the absence of vesiculæ seminales in this species. Protein and non protein nitrogen concentration is highest in the sperm rich fraction but on incubation of semen there is no appreciable increase in the level of non protein nitrogen, a fact which distinguishes dog semen from that of man. The level of hyaluronidase in the dog semen is very low (Swyer 1947).

## Semen diluents and storage

The chlorides of potassium, magnesium and calcium have been reported to increase the viability of dog spermatozoa *in vitro* when added to a diluent composed of 0.2 M sodium phosphate buffer, 0.5% sodium chloride and 1.5% glucose. Dog spermatozoa are delicate as compared to ram and bull since they are

easily harmed by washing. The motility of dog spermatozoa is depressed by diluent containing 0.02 M Carbonate buffer (Wales and White, 1958).

It is considered suitable to collect dog semen in three fractions and immediately after collection the ejaculates should be placed in waterbath at 35°C so as to effect gradual cooling to avoid temperature shock. Considerable difficulty is experienced in preserving dog semen for a longer period. Bederke (1933) observed that undiluted semen had a survival time of 21 hours and that dilution with blood serum reduced the survival time to half. He also observed that dextrose solution or in combination with sodium phosphate buffer has an unfavourable effect on the semen. Freiberg (1935) observed that the second fraction of the ejaculate which is the sperm bearing fraction can be preserved without dilution upto 24 hours. Brochart and Coulomb (1952) reported that the second fraction of the ejaculate diluted with egg yolk sodium citrate can remain viable and motile for a period of 4 days. The most successful diluent and preservative so far known for a dog semen is the heat treated pasteurised milk as recommended by Thacker and Almquist (1953). In preparing the diluent a small quantity of pasteurised milk is slowly heated at 92°C and kept between 92°C to 94°C for 10 minutes. It is then cooled to room temperature and used in the required quantities. Studies show that if the sperm bearing fraction of the ejaculate is diluted 1:8 with heat treated milk and the mixture stored at 4°C it will remain viable and fertile for several days. 50% motility was obtained at 120 hours.

Harrop (1956) conducted a series of experiments and proved that diluted semen can be conveniently transported to

Table 66 (Mann, 1964)

DOG SEMEN: COMPONENTS OF WHOLE SEMEN(S), SEMINAL PLASMA(P), AND SEMINAL FRACTIONS IN THE ORDER OF THEIR APPEARANCE AT EJACULATION (HARROP, 1960, HUGGINS, 1947, BARTLETT 1958, 1962)

		Unfractionated ejaculate	First fraction	Second fraction	Third fraction
Dry weight (%)	S	3.6-3.9	1.6-2.9	5.8-7.2	2.6-2.9
	P	2.3-2.7	1.5-2.7	2.6-3.6	2.2-2.7
pH.		6.1-7.0	5.8-6.6	6.0-6.6	6.1-7.2
Sodium (m-equiv./l)	S	56-124	146-195	180	136
	P	72-180	—	192	172
Potassium (m-equiv./l)	S	8.0-8.3	4.8-6.9	13.0	7.7
	P	7.9-8.2	—	12.4	7.5
Calcium (m-equiv./l)	S	0.4-0.9	0.3-2.2	0.4	0.2-0.6
Magnesium (m-equiv./l)	S	0.3-0.7	1.3-1.7	0.8	0.1-0.6
Chloride (m-equiv./l)	S	151.4	151-171	192	160
Bicarbonate (m. equiv./l)	S	1.1-6.4	1.7-2.5	2.3-5.4	1.7-2.7
Phosphate (mg. P/100 ml)	S	12.7-13.2	—	—	—
Total	S	10.9	11.2	36.0	1.5
Acid soluble	S	9.0	0.7	29.2	0.8
	P	1.0	—	—	—
Inorganic	S	0.64-0.67	0.16-0.43	0.91-1.33	0.35
Copper (mg/100 ml)	S	0.34-0.60	—	0.91-1.60	0.30-0.51
	P				
Zinc (mg/100 ml)	S	7.60-9.00	1.71-2.81	14.06-19.70	6.26-8.01
	P	7.13-8.65		16.06-20.74	5.88-9.39
Iron (Mg/100 ml)	S	0.02-0.09	0.01-0.04	0.04-0.10	0.01-0.02
Reducing substances (mg/100 ml)	S	20-21	3-22	14-25	6-11
Yeast fermentable sugar (mg/100 ml)	S	1.8-2.2	—	—	—



		Unfractionated ejaculate	First fraction	Second fraction	Third fraction
Fructose (mg/100 ml)	S	0.5-0.6	10.4-10.6	1.2-3.0	0.2-0.6
Lactic Acid (mg/100 ml)	S	11.0-30.0	5.8-10.7	20.6-41.2	7.9-21.0
Total nitrogen (mg/100 ml)	S	299-456	250-261	699	112
	P	307-452	—	659	403
Non-protein nitrogen (mg/100 ml)	S	26-39	27-39	61	31
	P	23-34	—	61	29
Protein (%)	S	1.71-2.61	1.46-2.01	3.99	2.38
	P	1.74-2.61	—	3.72	2.34

far off places and can be fit for insemination. Bendorf and Chung (1958) reported successful results with egg yolk citrate diluent and no adverse effects were noticed on long transportation. They also observed that semen preserved in egg yolk citrate kept its viability better than samples in which antibiotics were added.

#### Packing and Transport

This is done in the same way as for bull semen. Thermos flasks are used for transportation as usual. Sufficient care is taken to wrap cotton wool around the test tubes since dog spermatozoa easily die if they come in direct contact with ice. The samples packed in such a way and kept at 4°C can remain viable in the journey for about 72 hours. It is necessary to replace ice from time to time to maintain optimum temperature.

Deshpande *et al* (1970) on their work on insemination of 30 bitches resulted in conceptions to the extent of about 50%. One bitch was inseminated from semen imported from Great Britain (Hadi and Sane, 1968) resulted in fruitful conception. Semen sample diluted

partly in citrate and partly in milk from one dog was flown to Ceylon.

#### Insemination in Bitches

It was difficult to diagnose exact phase of oestrus, the day on which bitches were offered for insemination. A careful observation on a very large number of bitches is necessary to acquaint oneself with regard to the day to day changes during oestrus period. Under such circumstances it was difficult to offer any opinion and majority of bitches were required to be inseminated on the day they were presented irrespective of the fact whether they were in proper stage for inseminations. This is one of the reasons for low conceptions obtained in the present study.

The vulval tumefaction was observed in majority of the cases, but the quantity and quality of discharge did vary from case to case. Number of bitches offered during proestrus stage did show turgidity of vulval lips, vaginal mucus membrane moist and pink with serosanguineous discharge, such cases were recalled after a couple of days when it was observed that the vaginal

discharge was scanty or practically nil. Vaginal smears stained with Leishman's showed a typical cellular picture as described by Harrop. (1960).

In majority of cases neat semen was used for insemination in doses extending from 0.5 to 2.0 ml. One ml. glass pipette, with syringe attached is used for insemination.

A vaginal speculum may be slowly inserted and cervix properly located with the aid of headlight. The insemination pipette is then inserted through the cervix. The pipette can be inserted without the use of the speculum. In this procedure, the pipette is inserted between the lips of the vulva and pushed in the vertical direction until it has passed the vestibule. The pipette is then directed horizontally and passed through the cervix. The semen is then syringed directly into the uterus. It is beneficial to elevate the rear parts of the bitch following insemination.

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# Chapter 47

## Artificial Insemination in the Poultry, Ducks and Geese

During the recent years, Artificial Insemination has received considerable importance in developing poultry for commercial production. In breeding heavy and broad breasted turkey this method is being used extensively. The method is used supplementary to natural breeding to enhance fertility. Attention has also been focused to develop ducks in the Far East by artificial insemination (Onishi *et al*, 1955; Watanabe, 1957). In Formosa, the most important industries for meat production is the production of mule ducks namely intergeneric  $F_1$  hybrids between domestic ducks and Muscovy drakes, because of the rapid early growth of this cross. Artificial insemination has helped in alleviating the unsatisfactory fertility problem resulting from the impaired mating behaviour.

In Canada and U.S.A., Artificial Insemination method has shown good promise for goose production (Johnson, 1954; Merritt and, Gowe, 1956).

Particular emphasis has been stressed for the maximum use of progeny tested sires. The method proves very useful when the breeding male in a pen has to

be replaced due to some reason in the midst of breeding season. Under such circumstances, the simultaneous use of artificial insemination reduces the period of overlapping of chicks sired by the two males since it is quite natural that fowls may not be fertilized by the new male for some time.

In the poultry improvement plans, the necessity of importing birds of different breeds of poultry from other countries of the world is keenly felt. In such cases, instead of importing the birds which may bring known and unknown type of infections along with them, the best way is to import semen of progeny tested and disease free sires.

### Limitations

In-breeding is likely to occur to a great extent due to the maximum use of artificial insemination. The danger has to be avoided by careful planning and control. For commercial production, hens are usually kept in pens or battery cages. Excitement and over handling of the cage birds may reduce output from hens. It is observed that different breeds and strains of poultry may not respond to equal performance

in battery cages and pens (Gowe, 1956; Blount, 1951). Cooper (1955b) reported that the hatchability of all eggs set was in favour of the inseminated birds. Lowry *et al* (1956) observed that floor pullets laid more eggs based on the mean production of the survivors but on the other hand cage pullets showed low mortality and laid heavier eggs.

Bailey *et al* (1959) observed that cage birds were superior in the degree of their performance. Moultrie (1956) and Taran (1957) reported that the egg laying capacity of hen is not disturbed by insemination operations.

### **Reproductive System of the male and female**

The anatomy of the reproductive system of the cock is markedly different from that of other domesticated mammals. The location of the testis is within the body cavity. Such a location of testes would cause sterility in mammals. The duct system consists of paired epididymis and vasa deferentia. Accessory glands, such as seminal vesicles, Cowper's glands and prostate are not present in the cock. The vas-deferens does increase slightly in diameter prior to entering the cloaca and little semen is stored in this bulbus portion. In the cock, there is no functional counterpart of the mammalian penis. However a rudimentary penis is present in the cloaca. The paired vasa deferentia terminate in a double teat like structures known as papillae. These are clearly visible on sexual stimulation and it is from this region that the semen can be obtained.

The reproductive system of the hen is specialised for the production of a complicated egg. The upper region of

the oviduct receives the yolk, following ovulation from the ovary. Major portion of the oviduct is concerned with the secretion of material to form albumin and cell membrane. The cell secreting gland or the uterus is located about  $1/5$  of the distance of the oviduct from the cloaca. The short portion of the oviduct connecting the cloaca with the uterus is the vagina. This part is exposed when the oviduct is everted for insemination.

### **Management of the Cock**

Usually it is not possible to collect semen from a cock running free with the hens. In order to obtain best results, the cock should be caged individually. (Cages of  $50 \times 50$  cm, with wooden or wire floors are satisfactory. Before collection, the cock should at least be separated from the hens for about a day. Well trained cocks have to be handled quickly and carefully to avoid loss of semen due to premature ejaculation. Semen can be collected from cocks 3 or 4 times a week for indefinite periods without affecting the semen quality. However, there is good deal of individual variation on account of high or low plane of nutrition. For clear semen samples it is necessary to see that the droppings are not loose in order to avoid risks of contamination during collection. In case the droppings are loose, the best way is to feed the whole cereal grain and avoid mash and pellets.

### **Equipment**

A collection cup is required to obtain semen as it is emitted. A small glass funnel with paraffin sealing off the narrow outlet tube is found suitable. The outer tube is stuck into a cork or rubber

stopper. The distance from the stopper to the funnel lip may be adjusted to suit the operator's hands. A tuberculin syringe of 1 ml. capacity graduated in 100th serves an ideal purpose for transferring major doses of semen into the oviduct of a hen. Separate sterilised funnels should be used when semen has to be collected from a number of cocks.

### Semen Production

In the production of semen from the cock, there are two sets of factors (1) direct influences of diet, management and normal physiological processes which regulate the activity of the gonads and the reproductive tract in initiating the production of spermatid fluid and (2) factors which determine the degree of response of a male bird to the massage technique for collection of semen and the amount of semen that could be extracted from the erect ejaculatory ducts.

Feeding, rearing, housing and season does influence semen production. Parker and McSpadden (1943 b) observed that when food was restricted the volume of semen decreased and it contained fewer spermatozoa with reduced fertilizing capacity. The deficiency of Vitamins A and E have been shown to affect spermatogenesis and semen production (Rubio Paredes and Perez Garcia, 1959). Various drugs used for prevention of poultry diseases such as Enheptin or Entramin may affect spermatogenesis and semen production. Excessively high or low temperature adversely affects semen production, especially in the male Turkey. Kosin (1958) reported that the Turkey Spermatozoa display a tendency of reduced respiratory activity when the males were exposed to extremes of climatic changes. In the fowl, the production of semen in seasonal (Kamar and

Badreldin, 1959). In these species, the greatest semen volumes are obtained with increasing day length. Mating behaviour has seasonal expression although semen can be produced from a male during off season.

The volume of semen is dependent on the frequency of collection. The volume of semen obtained from Turkeys decrease in each successive ejaculate collected daily but the concentration of spermatozoa and the fertilizing ability do not vary significantly (McCartney *et al*, 1959). Males in the high mating frequencies yield smaller volume of semen on massage than those with low mating frequencies. Some males require frequent handling to stimulate them. The actual type of response induced by massage differs between breeds and between species of poultry (Wood Gush, 1960). In the Turkey, more prolonged erection is seen whereas in the cock it is momentary. Different degrees of pressure have to be applied to the erected copulatory organ of different cocks for extracting maximum quantity of semen. The amount of transparent fluid obtained from the erectile tissue in the cloaca by the massage collection method is variable and can cause a great variation in the density of spermatozoa between collections and periods (Lake, 1957a; Nishiyama, 1961).

### Collection of Semen

Collection of semen is done from the cock by massaging the dorsolateral lumbo-sacral region and also the abdomen which causes erection of the copulatory organ. On stimulation semen has to be expressed rapidly from the swollen ejaculatory ducts. The erection varies from bird to bird. In order to have the best response, the cock should be picked up quickly and gently from

its cage or pen and the tactile manipulations should be done immediately. (Fig. 159). In case, the initial excitement stage has passed, it is difficult to elicit reflex response to massage technique. Rough handling may inhibit the reflex and also increase the chances of urination and defaecation. Ejaculation is not spontaneous on massage and the quantity and quality of semen obtained is dependent on the degree of pressure that has been applied to the copulatory ducts. On stimulation, the ejaculatory ducts swell up and then it is possible to obtain semen with very light pressure of the fingers. In such a quick and gentle manner, chances of contamination with transparent fluid from cloaca, faecal matter and urine are rare. The cloacal fluid is also initiated on erection in response to massage. Opinions are still divided if cloacal fluid is a part of the seminal plasma or whether it is a separate entity having a different purpose (Lake, 1957-a; Nishiyama and Ogawa, 1961).

For collection of semen from Turkey-stag, the procedure is the same as that for the cock. After the tail fins are pushed forwards on the back of the bird it is advisable to apply digital massage over the large area of the skin above and below cloaca. Two operators are usually required one to hold the legs of the Turkey-stag and the semen collecting receptacle and the other to carry out massage.

In the gander and drake, the penis-like copulatory organ can be erected by massage. Technique is similar to that of the cock. The semen is released at the base of the protruded penis of the gander and it usually flows in a canal through the length of the organ. Semen can be collected from any point along the canal

or from the end of the organ. Care should be taken not to feed the male at least 6 hours before collection to avoid faecal contamination and bleeding.

For collection of semen from fowl, various receptacles are used. A glass or plastic filter funnel with a diameter of 3.75 cm. to 5 cm. is found suitable. The funnel may be warmed if necessary. For Turkey stags, the glass tube encased in a plastic water jacket is found suitable. Lorenz *et al* (1959) recommended "Stem cup" for collection of Turkey semen. Cooper (1955a) suggested suction method for collection of semen from cock. Allen (1957) devised a method of filter funnel with a calibrated tube attached to the stem with rubber tubing.

In general, it is observed that fowl spermatozoa are more resistant to temperature shock than those of the bull or ram. Therefore, if the semen is used within a short time after collection, it results in good fertility. It is best to use undiluted semen immediately after collection. Cock semen could be kept viable for four hours at 10°C (Schindler *et al*, 1955). A sharp drop in fertility was observed when Turkey semen was stored at either 10°C or 15°C for a period of 2 hours (McCartney *et al*, 1959). Harper (1955) recommended that Turkey semen should be used within an hour after collection, for higher fertility rate. Best fertility results have been claimed when neat semen is used for insemination within half to one hour after collection.

## SEMEN EVALUATION

### Volume and sperm concentration

Table 67 indicates the volume and density of spermatozoa in semen samples collected by massage method from various types of poultry (Lake, 1962).

Table 67

## SEMEN VOLUME AND DENSITY IN POULTRY

Breed	Location	Vol (ml)	Density ( $\times 10^6/\text{mm}^3$ )	Author
Cock	Egypt	0.35	0.03	Kamar (1958)
White Leghorn	India	0.34 to 0.43	0.9 to 3.8	Mukherjee and Bhattacharya (1949)
	Australia	0.25	3.1	Skaller (Personal communication to Lake, 1962)
	U.S.A.	0.75	2.2	Allen and Champion (1955)
Brown Leghorn	Scotland	0.4	7.0	Lake (1957-a)
Desi (Indian)	India	0.18 — 0.3	1.6 to 3.3	Mukherjee and Bhattacharya (1949)
Fayomi	Egypt	0.26	3.7	Kamar (1958)
White Baladi	-do-	0.32	6.53	-do-
Australorp	Australia	0.5	3.4	Skaller (Personal communication to Lake, 1962)
				Allen and Champion (1955)
New Hampshire	U.S.A.	1.26	2.2	Parker <i>et al</i> (1949)
	-do-	0.9	3.6	Allen and Cham- pion (1955)
White	U.S.A.	1.1	2.2	Parker & McSpald- den (1943a)
Wyandotie				
Rhode Island	U.S.A.	Apr. May	Apr. May	
Red		0.83	3.8	
Turkey				
Broad Breasted				
Bronze	U.S.A.	0.33	8.1	Parker (1949)
White Holland	U.S.A.	0.19	7.1	McCarthy (1949)
Duck-Osaka	Japan	Electrically		
		0.3	4.6	Watanabe (1957)
		Massage		
		0.2	0.25	



There is some decline in the quantity of ejaculate obtained from turkey tom with increasing age and hot weather (Smyth, 1969). Schindler *et al* (1957) and Kamar and Badreldin (1959) observed alterations in the motility and morphology of the spermatozoa. Saeki (1960) found that crooked neck spermatozoa in the cock semen resulted in low fertility.

### Hydrogen-ion concentration of Semen

The normal pH of the semen varies from 7.02 to 7.18. During long term storage, the pH of the semen changes due to the production of acid by-products of metabolism which may cause damage to spermatozoa. Wilcox (1958a) has shown that the hydrogen ion-concentration of undiluted cock semen reaches to pH 6.6 after 24 hours of storage at 10°C.

### Dilution and Storage

Several physiological solutions are used for diluting the semen. The diluted semen should be used within a short time. Glucose present in cock semen presumably originates in the transparent fluid from the cloaca (Mann, 1954). Wilcox (1960) has claimed good results by diluting cock semen in 1 to 10 proportion in phosphate buffer with antibiotics and kept at 10°C. Blackwood and Harris (1960) and Harris *et al* (1961) found that metabolic inhibitors particularly 2 ethyl-5 methyl benzi midazole, prolongs the life of the cock and Turkey spermatozoa in vitro. On account of the peculiar anatomical structure of the cloaca there is always a great risk of bacterial contamination of cock semen. Use of streptomycin in concentration of 50,100 and 200  $\mu$ g. per ml was found to be effective in checking

bacterial growth. Terramycin combined with dihydrostreptomycin in a dose 90  $\mu$ g each per ml of diluted cock semen have given best results (Wilcox and Shorb, 1958). The diluents for extending cock and Turkey stag semen are indicated in Table 68 (Lake, 1962).

### Insemination Technique

The most common method requires two persons. The first one to hold the hen firmly between the lower thighs with his left hand and at the same time holding the hen against his body. The right hand is used to expose and evert the oviduct. The thumb is placed above the cloaca and the fingers and palm on the abdomen below the cloaca. Eversion is effected by exerting pressure with the right hand. In fatty birds, additional pressure for eversion is necessary. In the non-layers eversion of the vagina is usually not possible. The vaginal opening is seen in the left side of the cloaca when oviduct is everted by pressure while as, the right side opening is that of the alimentary canal. The second operator inserts the Tuberculin syringe or pipette with bulb containing semen to the depth of about 2 cm. Before injecting the semen, abdominal pressure is released to avoid back flow of the semen out of the oviduct. When the oviduct returns to its normal position, the bird should be released.

Gabriel (1957) recommended technique requiring one operator. In this method the thighs of the bird are held tightly from underneath by the fingers and palm of the left hand. Holding the thighs in this position results in the partial crossing of shanks and exerts an upward pressure on the oviduct. The thumb of the left hand is used to apply pressure on the lateral sides of the abdo-

Table 68  
DETAILS OF THE USE OF DILUENTS TO EXTEND COCK AND TURKEY STAG  
SEMEN WITHOUT IMPAIRING FERTILITY

Diluent	Dilution rate	Maximum holding time (hr)	Holding Temp. (°C.)	No. of inseminations per week	Insemination dose (ml.)	Species	Author
Modified Ringers solution	3	1	Air	1	0.05	Cock	Bonnier and Trulsson (1939)
Tyrole solution	13	About 0.5-1	Air	1 or 2	0.2	Cock	Allen and Skaller (1958)
Modified Ringers solution	3	4	10	1 per 6 days	0.1	Cock	Schindler <i>et al</i> (1955)
Ringer Locke solution	Do	Do	Do	Do	Do	Do	-Do-
Whole pasteurised milk	Do	Do	Do	Do	Do	Do	-Do-
Glutamate containing fluid	3	0.5	15	1	0.05	Turkey	Lake (1962)
	2	7	2	1 per 6 days	0.1	Cock	Lake (1958)
Phosphate buffer containing albumin, fructose and antibiotics	2	8	10	1	0.1	Cock	Wilcox (1960)
Tyrosine solution	3	0.25-1.75	3.5	1 per 3 weeks	0.03	Turkey	Van Tienhoven and Steel (1957)

men. This combination gives effective pressure and the oviduct is forced out. The semen is then deposited by the right hand. About 200 hens per hour may be inseminated by adopting this method. It is advisable that all hens should be palpated prior to inseminations. In case there is any hard egg in the uterus, insemination should not be done. The best time to inseminate is during late hours of the day after the laying is over. The standard recommended dose of neat semen per hen is 0.1 ml. A dose less than 0.05 ml. will result in low fertility.

In one man's technique for insemination, the hen is held in the left hand with its right leg between the index and second finger and its left leg between the third and fourth finger. The middle finger may also be employed to apply pressure to the lower abdomen. The right hand is used for everting the oviduct and on eversion enough pressure should be applied with the middle finger of the right hand to maintain the eversion. The right hand which is thus free should be used for insemination (Mueller, 1949).

Technique recommended by Moultrie (1956) can speed up the rate of inseminations to about 40 per hour. One operator grabs the hen by hocks with his right hand and pulls the feet and shanks through the cage door leaving the body of the bird resting on the cage floor. In order to exert some pressure on the anterior part of the abdomen, the legs must be held firmly together. The left hand is then used in everting the oviduct. The second operator then injects the semen.

For maximum fertility, a hen should be inseminated at seven day intervals. The average duration of fertility in the

chicken is between twelve to fourteen days. Programme for weekly inseminations is necessary for hens of lower fertility. With one insemination only a fertility rate of 85 per cent may be expected for a period of ten days. Duration of fertility is on the decline as the age advances and as such insemination every four or five days is desirable.

## ARTIFICIAL INSEMINATION IN THE TURKEY

The reproductive system of the Turkey closely resembles that of the chicken. It is difficult to obtain semen from a Turkey-Tom-stag as compared to Cock. The operator has to acquire plenty of skill and practice. Equipment required for collection is the same as that in the cock.

### Management of the Turkey-Tom

It is on the same lines as in poultry. The Toms should be segregated from the females at least for 24 to 48 hours before collection of semen. Turkey-Toms on account of their large size can rarely be kept in the cages. If cage is used, it should have a solid floor and not of wire which may cause injury to the feet. In order to prevent undue excitement place it in a very little light to facilitate catching of the toms. In case there is over excitement toms struggle a lot and then it becomes difficult to stimulate them. Excitement also results in frequent contamination of the semen with watery faecal matter. It is advisable to withdraw water and feed at least for about 4 to 6 hours prior to collection. However, in hot weather water should be given sparingly.

During the season, semen can be collected from Turkey-Toms as frequently

to avoid bleeding. Faecal contamination can be reduced by withholding feed at least 6 hours prior to collection. By the electro-ejaculation technique better results have been claimed than the massage method (Watanabe, 1957). In the technique followed by him, one electrode is placed on the skin in the sacral region and the other one inserted in the vent. Then an alternating current of 30 volts and 0.06 to 0.08 amperes is applied for 3 seconds at 5 seconds intervals repeating 3 to 5 times.

#### Evaluation of Drake and Gander Semen

The semen of Drake and Gander is of more watery consistency and of faint colour as compared to that of chickens and turkeys. The sperm concentration is very variable. Johnson (1954) in his studies on 6 Ganders recorded that their average volume of semen ranged from 0.05 to 0.60 ml. per collection. The quantity and quality of semen was best during the breeding season.

The average yields of semen from common drakes have been reported as 0.23 ml. (Watanabe and Sugimori, 1957) and 0.32 ml. (Onishi *et al.*, 1955) by the manual massage method. By the electro-ejaculation technique, an average of 0.33 ml has been reported by Watanabe (1957). A larger average of 0.82 ml. of

#### Dilution, preservation and storage of Avian Semen

In the poultry breeding programme by Artificial Insemination, dilution of avian semen does not seem to be favourable. It appears from the studies that avian semen is more susceptible to the diluents as compared to dilution of semen of other domestic animals. The egg yolk citrate and phosphate buffers used satisfactorily as diluents for bull semen, do not appear to be satisfactory diluents for chicken semen. Egg albumin is found to be a poor diluent even though sperm life is sustained for prolonged periods in the upper region of the avian oviduct where albumin is secreted. However, certain albumin proteins have shown better effects when added to diluents (Lorenz and Tyler, 1951).

soft area of the abdomen. The assistant holds the hen in his lap and applies pressure on the abdominal area while the inseminator holds the legs. Immediately on eversion of the oviduct, the inseminating syringe should be inserted to a depth of approximately 2.5 cm. An easier and faster method than this is the one in which the hen is placed head first through the helper's legs and held in position by the helper's thighs. The holder may apply sufficient pressure with his leg to keep the hen in position and at the same time apply abdominal pressure with his hands. The inseminator should hold the legs of the hen by his left hand to avoid struggling during insemination. It is experienced that the devices which hold the female bird nearly parallel to the ground are superior to those in which the bird is held in a perpendicular plane in which position there is every likelihood for the watery droppings to run into the oviduct along with the seminal fluid.

Insemination should be done during late hours in the afternoon. Abdomen should invariably be palpated to confirm that there are no eggs in the passage. Decreased fertility will result in case inseminations are done when hard eggs are within the uterus. If Turkey hens are to be inseminated before they start laying their first eggs, a membranous tissue known as the hymen will be observed across the opening of the oviduct. In natural mating, it is broken by the treading of the tom or it may get broken due to pressure during the passage of the first egg. The hymen can be broken easily with the end of the syringe.

Several types of syringes are used for insemination. The dental syringe seems to be the popular one.

A dose of 0.025 to 0.035 ml. of semen per turkey hen is said to give good results. Late in the breeding season it is preferable to increase the dose to 0.05 ml. During early part of the season, fairly good fertility can be obtained between 40 and 50 days. Gradual decline will be noticed as the season progresses. Fertility upto 85% is possible over a 30 day period following insemination early in the breeding season. To begin with, it is better to inseminate hens every 3 or 4 weeks. By the third month the interval should be shortened to 2 or 3 weeks and even less than this if the season extends to five months (Smith and Jeffrey, 1960).

## DUCKS AND GEESE

The reproductive organs of ducks and geese are very similar. In some respects both these species differ from those of chicken and turkeys. The major difference is in the presence of a well defined penis in the Drake and Gander. During the breeding season the penis becomes greatly enlarged. The water fowl extrudes its penis just prior to natural mating. In the ducks and geese the oviduct cannot be artificially everted, therefore, a different technique for insemination is adopted.

### Collection of semen from the Drake and Gander

It is similar to that for chicken. Massage method has been recommended by Johnson (1954). Two men technique is found useful. The semen is released at the base of the penis and normally runs in a canal throughout the length of the organ. Semen may be collected from the end of the organ. Gentle handling of the bird is necessary

to avoid bleeding. Faecal contamination can be reduced by withholding feed at least 6 hours prior to collection. By the electro-ejaculation technique better results have been claimed than the massage method (Watanabe, 1957). In the technique followed by him, one electrode is placed on the skin in the sacral region and the other one inserted in the vent. Then an alternating current of 30 volts and 0.06 to 0.08 amperes is applied for 3 seconds at 5 seconds intervals repeating 3 to 5 times.

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Use of chemically pure salts is recommended and the pH is adjusted between 6.6 to 6.8 (Bogdonoff and Shaffner, 1954).

### Turkey semen

Earle's solution is used for dilution of Turkey semen although it results in 15 per cent less conception rate as compared to insemination by neat semen. This is also inferior to results claimed by Van Tienhoven and Steel (1957) who used Tyrode's solutions having a pH of 6.7 with homogenised, pasteurised whole milk. When such a diluted semen was used in the proportion of 1:1 and 1:3 the three weekly fertility was practically equal to that for the undiluted semen. Fertility was not affected due to variations in time between dilutions and inseminations from 13 to 106 minutes.

### Duck semen

Satisfactory fertility resulted by use of diluted drake semen at the rate of 1:10 with saline solution (Watanabe, 1957). It is observed that drake sperms are found to be more resistant to salt solutions than cock sperm.

### Storage of Semen

For preservation of avian semen, very satisfactory methods have not as yet been devised. Available reports show that there is a tendency for decrease in fertility by the use of semen stored beyond two hours at various temperatures. Garren and Shaffner (1952) recorded that storage reduced the duration of fertility in all stored groups. However, the rate of fertility was improved when the dose was increased from 0.10 to 0.20 ml and simultaneously when the interval

between inseminations was also increased. In case, the whole semen is to be stored for more than an hour, the temperature should be reduced gradually to 14°C-9°C. Wilcox's method for preservation of semen involves the use of two different diluents. One for dilution and storage and the other for reconstitution prior to insemination.

1) Diluent 'A' is prepared in 1 litre of distilled water by adding 16.34 gm of  $\text{Na}_2\text{HPO}_4$ , 5.16 gm of  $\text{NaH}_2\text{PO}_4$ ,  $\text{H}_2\text{O}$ , 100 mgm of terramycin and 1000 mg of dihydrostreptomycin. Semen is diluted at 1:10 ratio and stored at 10°C. Following storage, the sample is centrifuged at 1000 R.C.F. for 10 minutes and the clear fluid is poured off leaving only the sperm. 2) Diluent B is prepared by mixing 1 ml. of solution containing 60 mg. of fructose per ml. to 14 ml. of a buffer solution containing 5.16 gm.  $\text{NaH}_2\text{PO}_4$ ,  $\text{H}_2\text{O}$  and 16.31 gm. of  $\text{Na}_2\text{HPO}_4$  per litre. This is added to the sperm fraction in an amount to constitute the original volume of the semen sample before the first dilution by mixing well. Insemination should be done immediately.

### STORAGE OF TURKEY SEMEN

The storage methods are similar to those of cock semen. Undiluted turkey semen is observed to be more resistant to storage than cock semen. Turkey semen stored at 14°C for 2, 4 and 6 hours resulted in fertility rate of 93, 80, 72 and 55% respectively (Carter *et al.*, 1957). No significant adverse effects of holding time on hatchability were observed. Harper (1955) observed that in the beginning of the breeding season, turkey semen could keep well as long as 4 hours at 13°C to 15°C without affecting ferti-

lity. However, towards the end of breeding season, holding time of even one hour resulted in a decrease in the rate of fertility. Fairly good results have been claimed by using turkey semen for inseminations within an hour after collection at holding temperature between 24°C and 29°C.

### Evaluation in relation to fertilizing ability of Avian Semen

Neither volume nor sperm concentration is related to fertility except when the total number of spermatozoa used falls below 100 million. Positive relationship exists between high initial motility and good fertility. In scoring the motility, samples showing rapid swirling should be scored as the highest and samples showing individual cell movement scored as poor. Parker *et al* (1942) observed decrease in fertility with increase in the number of abnormal spermatozoa.

### Insemination Technique in the Duck or Goose

In these birds, the semen has to be deposited directly into the cloaca since it is not possible to evert the oviduct. The pipette should be inserted into the vent deep enough or insemination may be done by using tuberculin syringe. Use of speculum is also recommended to locate the oviduct.

A dose of 0.05 ml of Gander semen is recommended for insemination in geese (Johnson, 1954). A dose of 0.30 ml. of whole semen diluted with 1:10 of saline solution or a dose of 0.03 ml. of whole semen per insemination is recommended by Watanabe (1957).

An average interval of 9.7 days between inseminations has been recommended by Johnson (1954). However,

following the laying of first fertile egg, he recommends an interval of 6.1 days. Johnson (1954) recorded that chickens, turkeys and ducks are very fertile on the second day following insemination, whereas in the goose the non-fertile period following insemination is as long as that of 3.6 days. It is thus clear that for higher fertility rates geese should be inseminated every week. Watanabe and Sugimori (1957) observed that the common ducks have an average duration of fertility of 8 days. In general it can be said that in the usual breeding programme for geese, insemination should be done every 5 to 7 days.

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# Chapter 48

## Deep Freezing of Semen

Several decades ago many reports have been published about deep freezing of cells and organs of plants and animals. The findings were summarised by Luyet and Gehenio (1940), in the book "Life and Death at Low Temperature". The main idea with deep freezing was to arrest the processes of life, so that cells and organs could be kept for a very long time. Jahnelt (1938) found that by deep freezing testicular tissue of rabbits, sperms could survive freezing to  $-192^{\circ}\text{C}$ . The same thing was shown by freezing human semen to  $-79^{\circ}\text{C}$ ,  $-192^{\circ}\text{C}$  and  $-271^{\circ}\text{C}$ . In 1938, Luyet and Hodapp showed that if frog semen, dehydrated with a strong sugar solution spread out to a thin film on a cover slip and frozen in liquid air, could show relatively good motility after thawing. However, attempts to use the same method by deep freezing bull and human semen failed to succeed. Rostand (1916) found that frog semen treated with glycerol could stand temperature of  $-1^{\circ}\text{C}$  and  $-8^{\circ}\text{C}$  much better than uprepared semen. In 1919 Polge, Smith and Parkes, who did not know the work of Rostand by a pure chance found, that by adding 15-20% glycerol to a fowl sperm solution that it was possible to maintain very good motility after freez-

ing to  $-79^{\circ}\text{C}$ . Later on it was shown that the resistance against deep freezing is quite different in different species. It is known that bull semen cannot withstand a sudden drop in temperature so called *temperature shock*. For that reason Smith and Polge (1950) cooled the glycerol treated bull semen slowly to  $-79^{\circ}\text{C}$  and got a much better survival of the semen than that by rapid cooling. The first inseminations with deep frozen semen were performed by Stewart (1951) in co operation with Polge and Rowson. Out of 5 inseminated cows one became pregnant. Further work by Polge and Rowson (1952 a) indicated that the best results were obtained if the bull semen was first diluted with a diluent of 3.92% sodium citrate containing 50% egg yolk and cooled to  $+5^{\circ}\text{C}$  in 3-4 hours. At this temperature the semen was diluted 1+1 with a dilutor of the same sodium citrate solution and before containing 20% glycerol and allowed to equilibrate over night. On the following day, 1-ml portions were transferred to small glass ampoules which were sealed and cooled slowly over 45 minutes from  $+5^{\circ}\text{C}$  to  $-79^{\circ}\text{C}$ . Thirty eight cows were inseminated with semen treated in this way and of these cows 30 were found to be in calf.

Subsequent development has been the introduction of new freezing techniques, improved semen dilutors and additives, such as skimmed milk and sugar. Further new methods have been introduced viz. (1) *Pellets* concentrated semen suspension in sugar dilutor, frozen on a dry ice block (2) *Payettes* semen frozen rapidly in polyvinylchloride (PVC) straws of small volume and diameter.

### Physiology of Deep Freezing

Among the numerous attempts to preserve spermatozoa, deep freezing to arrest their metabolic processes is at present accepted as the only practical method in long term preservation of bull semen. The general principle for deep freezing of semen is to dilute the semen with an extender containing glycerol. After equilibration, the suspension is frozen according to special rules and thereafter stored either by using dry ice ( $-79^{\circ}\text{C}$ ) or liquid nitrogen ( $-196^{\circ}\text{C}$ ) as the cooling medium. Resistance of sperm to deep freezing varies with different species of animals. Bull spermatozoa are very sensitive to thermal shock. The freezing rate is thereafter an important factor even with the presence of glycerol. Formation of ice crystals damage

more compressing the sperm. In glycerolized semen on the other hand the ice crystals were much smaller and between the crystals were channels where the sperms were lying without being compressed.

As intra and extra cellular water crystallised as ice, the salts get concentrated in the residual fluid which becomes progressively more hypertonic. Thus the cells are subject to severe osmotic stress and in particular to increased concentration of electrolytes when the water is freezing out. Lovelock and Polge, (1954) were able to correlate the amount of damage sustained by cells frozen to various temperature with their sensitivity to hypertonic salt solutions at  $0^{\circ}\text{C}$ . According to Lovelock and Polge (1954) the first action of glycerol is to prevent the fatal increase in the concentration of electrolytes in the suspending medium which would otherwise take place as water crystalizes. Glycerol has the ability to attach to itself a considerable quantity of water, which is then not available to form ice but can still act as a solvent. The use of glycerol, therefore enables freezing to be carried out slowly enough to avoid thermal shock without exposing the cells to lethal concentrations of electrolytes.

(1958) found highly significant difference in conception rate between the first and second ejaculates i.e. 50.2% and 65.4% N.R. respectively.

### Dilutors for Deep Freezing

Polge and Rowson (1952-a) in their first deep freezing experiments used 3.92% sodium citrate solution with a final concentration of about 20% egg-yolk and 10% glycerol. Cragle and Myers (1954) and Cragle *et al* (1955) obtained the best results using 2.4 to 3.3% sodium citrate solution while Van Demark and Kinney (1954 a & b) found 2.9% sodium citrate solution to be the best.

Dunn and Hafs (1953) used milk with a final concentration of 10% glycerol as a dilutor for deep freezing of bull semen. Compared with citrate yolk dilutor, milk gave slightly better survival. O'Dell and Almquist (1954) used heated skim milk (92°C for 10 minutes) or heated homogenized milk. They used varied glycerol concentration time for the second dilution, equilibration time and thawing temperature. Best results were obtained by adding the second dilutor in 3-5 portions with about 10 minutes interval. Glycerol concentration 10% and 13% was better than 7% and even better than 4% and 16%. Thawing temperatures of 15°C or 40°C were superior to 22°C.

Kinney and Van Demark (1954) obtained best results between 16 to 24 per cent egg yolk by using a sodium citrate buffer with 7% glycerol. Saroff and Mixner (1955) found that 20% egg-yolk gave best survival when sodium citrate buffer with 7% glycerol was used. They further showed that an interaction occurred between the level of egg yolk and glycerol and suggested that the egg yolk

reduced the effectiveness of glycerol and therefore, with a high egg yolk percentage a higher glycerol percentage was required. Dunn and Hafs (1953) showed better sperm survival by using egg yolk at both first and second dilution. Hafs and Elliott (1954, 1955) used egg yolk in both diluting fractions, but with a higher citrate concentration in the glycerol containing fraction. In a field trial on about 700 cows in each group this method was found superior by more than 8 per cent in conception rate compared to the normal method with the same citrate concentration in both fractions.

Polge (1953) showed that bull semen with-stood freezing better with increasing glycerol concentration from 1% to 20% but the damage to the sperm before freezing increased rapidly with higher glycerol concentrations above 10%. Miller and Van Demark (1954) used, 2, 4, 6, 8 and 10% glycerol at the final dilution in sodium citrate egg yolk dilutor. Six per cent and eight per cent glycerol gave better survival while 2 per cent and 12 per cent gave poor results. Erickson *et al* (1954) obtained best recoveries with 7% glycerol using a 2.9% citrate solution. Cragle *et al* (1955) and Saroff and Mixner (1955) obtained better result with 7.5% and 7.6% glycerol respectively to yolk citrate dilutor. Rapid addition of glycerol to the diluted semen sometime causes severe damage to the sperms. Most workers favour slow addition of the glycerol in portions over a period of 30 to 60 minutes. The physically most satisfactory method is to introduce the glycerol by dialysis using a cellophane dialysis sac (Rowson, 1956).

Experiments by Graham *et al* (1958) showed no significant difference in the

fertility between deep frozen semen, when the glycerol was added in the initial dilutor at 30°C or semen glycerolized in two stages at 5°C, 10°C or 20°C. Stewart (1961) reported no drop in fertility with this method compared to addition of glycerol at +5°C. Settergren (1962) added the glycerol at room temperature ten minutes after primary dilution which gave the same survival in semen stored upto one month as glycerolization at 5°C.

Inclusion of sugar in the diluent has been reported to give better survival of semen after freezing (Emmens and Martin, 1957). In recent experiments, Nagase and Graham (1964) used sugar dilutors for freezing semen in a concentrated pelleted form. Glucose, lactose, raffinose, arabinose or combinations of these were used. There were no statistically significant differences between the dilutors in fertility trials but sugar dilutors were significantly better than whole milk.

The addition of antibiotics to semen prior to freezing has been tried. Erickson *et al* (1954) using streptomycin and penicillin in various combinations and singly found slightly better sperm revival than without antibiotics. Sulphanilamide has been found to be deleterious (Dunn *et al*, 1953).

Graham *et al* (1972) used Zwitter ionic buffers titrated with bases. They used Tris at pH 7.0 and reported higher motility of spermatozoa in this diluent. Simmet (1975) used Tris glycerol diluent prepared in concentrated form (Triladyl) to be diluted later with egg yolk and distilled water in proportion of 25:25:75 for diluting bovine semen at room temperature and directly filling

and sealing, and then freezing after equilibration at +5°C. This has given improved fertility over Cassou's method of freezing. Anderson (1974) found no significant differences in fertility rates of Cassou straws and Simmet's landshut Mini-tubes. Roussal *et al* (1972) used Mono sodium glutamate solution for freezing bull spermatozoa. Lopatko and Tyupina (1972) used raffinose glycerol egg yolk as most suitable medium for freezing of bull semen.

### Equilibration time

Polge and Rowson (1952 a) suggested in their first experiments the equilibration time to be 18 hours. Cragle and Mayers (1954) found the optimal equilibration time to be 14.9 hours while Dunn *et al* (1953 c) obtained best viability with five hours equilibration. O'Dell and Almquist (1957) found little influence on the survival after deep freezing from different lengths of equilibration time (1½, 4 or 18 hours). An explanation for some of these different opinions about the equilibration time may be due to the fact that the cell membrane appears to become more permeable with increasing age of the semen (Rowson, 1956). Another explanation is offered by Polge (1957) who found that semen withstood relatively slow freezing within critical period from -15°C to -25°C much better after a long equilibration than after a short one.

Stewart (1961) reported the same or some what better results with 5½ hours equilibration than with 24 hours. Settergren (1962) found the same survival rate in semen frozen after four hours equilibration as after 16 hours. Nagase and Nida (1964) started freezing semen four

to six hours after collection by pellet method

Martin (1966) used equilibration time of 2 to 18 hours and found equilibration of 8 hours to be better than 2 hours or 18 hours. Kalugin (1971) studied the effect of equilibration time from 0, 2, 4, 6, 8, 10 and 12 hours for recovery rate and found that 10 hours equilibration was better. Weitze (1973) found reduction in equilibration time reduced the motility. Wiggan and Almquist (1975) designed a factorial experiment for determining optimum equilibration and thawing temperature and reported that half an hour equilibration and 95°C thawing temperature for 7 seconds was most optimum from the point of view of intact acrosomes.

Additions of sugars like fructose and arabinose help to reduce equilibration time. There are many conflicting reports regarding the length of equilibration time and its effect on spermatozoal survival rates. It has been reported that equilibration as short as 1 minute could be employed for successful freezing (Jondet 1972). The earlier recommendations to equilibrate semen for a period of 15 to 20 hours is not generally followed now. However, it is evident from the above that there is no fault in post thaw motility or fertility because of reduction in equilibration time from 1 to 6 hours.

### Packaging of semen

The method of ampouling the semen varies considerably. Most common is the use of all glass ampoules sealed on the flame as packaging with rubber corks

may cause leakage in such ampoules (Macpherson, 1954, Van Demark and Kinney, 1954 b). Dunn *et al* (1954) used polythene bulbs for freezing semen, but Musgrave and Heath (1957) found low fertility with semen frozen in plastic bulbs as compared to glass ampoules. Graham and Erickson (1959) found in three different experiments better fertility of semen frozen in glass ampoules than the semen frozen in plastic vials (65.4% and 58.9% non return rates respectively). Brugman and Schmidt (1958) found no significant difference between plastics and glass ampoules. Even the size and shape of the ampoules are important for the result by deep freezing of semen. Bane and Settergren (1972) found that by freezing the same volume of semen in vials with different diameters the percentage of surviving sperms slightly increased with decreasing diameter of the vials. In glass tubes the outer part of the semen showed better motility after freezing and thawing than the central part. Cassou's 'french straw' most commonly known today are used in whole of the European continent as well as America, Canada and many of the developing countries. The 12 ml polyvinyl chloride straws of Sorensen were improvised to contain 0.5 ml (Medium French Straw) and 0.25 ml by further reducing the diameter (Mini Straw) by Cassou (1964-1968). It has been noticed during freezing experiments that the medium straws of Cassou had better surface area and weight combination and semen frozen in it had higher fertility rate as compared to ministraws which had same surface area but less weight (Simmet 1975). Simmet (1972) has used steel and glass balls to seal the french medium straw without plug i.e.

90 mm. and found it most suitable for freezing semen. The balls sealed straw perfectly well hence no leakage and no trouble in insemination. He further reduced the size of minitube to 65 mm. to contain 0.3 ml. of semen volume. The sheath for insemination required special system by which the ball and straw could be retained in the sheath allowing only semen to enter the uterus. This method is known as 'Landshut' method of packaging and freezing semen. It is fully automatic. The straws are 65 mm (half of Cassou medium straws) and are sealed by steel and glass balls. The semen packaged in it is 0.3 ml. These straws are known as 'Mini Tube' special printing machines are used for printing minitubes. The printing is very fast and clean. The filling and sealing machine fills and seals 4000 straws in 1 hour.

#### Advantages of Minitubes

1. It allows room temperature filling and sealing by use of special diluent — Tris-egg yolk with 6% glycerol.
2. The volume of semen is not much changed from that of French Medium Straw but has increased the productivity to double. The size of goblets is reduced and more handy.
3. It is fully automatic and more hygienic, through use of ball sealing and less direct handling of straws.

#### DEEP FREEZING METHODS

Deep freezing can be done by two methods—

- (i) Slow freezing (Ampoule)
- (ii) Instant freezing (Pellet and Straw)

Slow freezing in ampoule method is going out of date because of its involvement with varied freezing rate. Most commonly used method is Instant freezing. In Europe, America, Canada, Australia and other developed countries vapour freezing is followed while Japan, East Germany and some part of Finland and West Germany are still using pellet freezing.

#### Freezing procedure

The ampoules or tubes are placed in an alcohol bath at  $5^{\circ}\text{C}$  temperature at the beginning of freezing. For practical use, the most convenient freezing medium is solid carbon dioxide, which has the temperature of  $-79^{\circ}\text{C}$ . By adding small pieces of carbon dioxide the temperature is successively lowered. In the mechanical refrigerators it is possible to lower the temperature automatically at required rates. Polge and Rowson (1952 a) suggested a freezing rate of  $1.2^{\circ}\text{C}$  per minute from  $+5^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  and  $3.5^{\circ}\text{C}$  per minute from  $-15^{\circ}\text{C}$  to  $-79^{\circ}\text{C}$ . Erickson *et al* (1951) compared different freezing rates within the temperature range from  $-15^{\circ}\text{C}$  to  $-30^{\circ}\text{C}$  at the rate of  $3^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  per minute for milk dilutor. Best sperm survival was obtained with  $2^{\circ}\text{C}$  to  $5^{\circ}\text{C}$  per minute in yolk citrate dilutor and  $2^{\circ}\text{C}$  per minute in the milk dilutor. Saroff and Mixner (1955) used a freezing rate of  $1^{\circ}\text{C}$  per minute from  $+5^{\circ}\text{C}$  to  $-15^{\circ}\text{C}$  and 3 to  $11^{\circ}\text{C}$  per minute from  $-15^{\circ}\text{C}$  to  $-75^{\circ}\text{C}$ .

In storage trials for 0, 2, 4, 8, 16, 32 weeks O'Dell *et al* (1955) found the optimum freezing rate for semen diluted in fresh skim milk or homogenized milk



to be 1°C per minute from +5°C to -15°C, 5°C per minute from -15°C to -50°C and rapidly from -50°C to -79°C. The optimum rate for semen diluted in egg yolk citrate was 3°C per minute from +5°C to -15°C and 5°C or 10°C per minute from -15°C to -50°C.

Rapid freezing by placing the ampoules directly into crushed ice was reported by Bruce (1956) to give a non return rate of 56.7% compared to 63.0% with the slow freezing method. Polge and Jacobsen (1959) reported similar motility with fast and slow freezing methods. Jondet (1964) described a rapid method for freezing of semen in plastic straws. The straws were held above the surface of liquid nitrogen in Linde storage containers (LNR 25 and LNR 185) and the temperature was lowered to -196°C in three minutes.

A method to freeze semen diluted 1:4 in sugar egg yolk glycerol directly on dry ice has been described by Nagase and Niwa (1964). The concentrated semen suspension which is stored as pellets with a volume of 0.03 ml to 0.2 ml is further diluted at the thawing prior to insemination.

Mohanty (1973) studied the effect of freezing methods and extenders on the progressive motility of frozen bovine semen. He did not find any significant difference among the freezing methods (dry ice alcohol and liquid nitrogen). But among the diluents within the freezing methods there was slightly significant difference suggesting that the diluents have some significant effect on the freezability of semen samples as far as the post thawing progressive motility is concerned.

### Slow freezing

Slow freezing was carried out in alcohol CO<sub>2</sub> ice bath. The temperature of alcohol CO<sub>2</sub> ice bath is brought to +4°C the same as that of equilibration. The equilibrated semen ampoules are then immersed in alcohol CO<sub>2</sub> ice bath. The temperature is recorded with the help of frozen semen thermometer (+30°C to -200°C). The CO<sub>2</sub> ice blocks are put one by one slowly so that the temperature goes below +4°C at a definite rate. The rate is as follows:

- +4 to -10°C at the rate of 3°C/minute
- 10 to -20°C in one minute
- 20 to -30°C at the rate of 5°C/minute
- 30 to -40°C at the rate of 10°C/minute
- 40 to -79°C fast

The ampoules get frozen. They can be preserved in alcohol CO<sub>2</sub> ice bath or can be transferred to liquid nitrogen container in canisters for long term preservation. This process also can be achieved by automation using biological freezers and freezing tunnel. Where the air circulation is forced over liquid nitrogen to give controlled vapour around ampoules. The temperature is reduced by potentiometer recorder. The freezing rate can be controlled and slow freezing can be achieved by freezing in conventional CO<sub>2</sub> alcohol bath and forced controlled vapour freezing in biological freezing or by freezing directly in crushed CO<sub>2</sub> ice (Jakobsen, 1956; Bruce, 1956 and Bhosrekar 1977).

### Pellet freezing

This was used by Japanese workers Nagase and Niwa (1964) using sugar

ns, 7.5% glucose, 10.5% lactose or raffinose containing 5 to 5.5% of medium with equilibration of 5 to 10

The semen was placed in small droplets of 0.5 to 0.2 ml using micro-syringes. The droplets are placed for 10 minutes on dry ice, then transferred into containers and stored in liquid nitrogen. Nagase *et al* (1963), Nagase and Niwa (1961) and Nagase and Niwa (1961) reported higher fertility rates with frozen semen as compared to ampoules. Adler *et al* (1968) found 15% of spermatozoa in use of pellets as compared to straws. Seifert and Beller (1967) developed less expensive method of pellet freezing using liquid nitrogen and solid CO<sub>2</sub> ice. The pellets can be stored on Nylon thread which is put on an ice block. It will help in tagging and identification.

### W-Vapour freezing

Andersson (1964) and Cassou (1964 and 1965) described the methods for freezing of medium straws 0.5 ml. The equilibrated straws after drying in cold chamber are arranged on freezing ramps and the help of straw spreader. The straws are then placed on grill in 250 ml liquid nitrogen container (LR 250). The grill is placed at a depth of 30 cm. Below the top trim of the container and liquid nitrogen level is kept at the grill. The freezing racks have the height of 10 cm and at the level of straws the vapour temperature is about -175°C to -180°C, within 8 to 9 minutes the side temperature of the straws reach -140°C. The freezing rate varies with the depth of vapour in the tank, liquid nitrogen level in relation to the straws and number of straws frozen at a time. Volume of semen and type of diluent.

Approximately 600 minitubes or 300 medium straws can be frozen at a time on a grill in 250 litre container. After allowing 10 minutes the straws are collected with the help of special forceps and placed in goblet. The goblet is then immersed in liquid nitrogen. The temperature of liquid nitrogen is -196°C (Bhosrekar, 1977). Bull spermatozoa can survive the temperature of liquid helium i.e. -296°C (Nishikawa, 1972). Almquist and Wiggin (1973) investigating the different methods of freezing have reported that best revival rate was in single straws arranged in racks and horizontally frozen in static vapour of liquid nitrogen.

### Handling of frozen semen

The basic principle of any cold storage is that the cold chain must not be broken. Even at frozen state the temperature fluctuation from -79°C to -196°C and vice versa adversely affects the fertilizing power of spermatozoa (Kalev *et al*, 1974). So deep frozen semen should be transferred under liquid nitrogen. Almquist and Wiggin (1973) have reported that any condition which will cause rise in temperature from -180°C to -120°C will affect the spermatozoal life under storage.

### Storage of frozen semen

Frozen semen is stored at

- (i) -79°C by using solid CO<sub>2</sub> (dry ice) and alcohol
- (ii) -190°C by using liquid air
- (iii) -196°C by using liquid nitrogen
- (iv) -296°C by using liquid helium.

Storage of frozen semen in liquid nitrogen is the most convenient and accepted method all over the world. Soon

after the temperature of straws reaches about  $-140^{\circ}\text{C}$  the straws are collected by pre-cooled forceps and transferred into pre-cooled goblets. These are then immersed in liquid nitrogen and goblets are then kept in already identified canisters under liquid nitrogen. The semen should be stored for one month in the container in which it is frozen, then it should be transferred to another long term storage container to safeguard against the spread of Foot and Mouth disease. This is very essential from quarantine point of view that Foot and Mouth virus appears in semen much before the bull suffers clinically from the disease since incubation period is about 8 to 10 days. It has been shown that F and M virus could be preserved under liquid nitrogen as like sperms.

There are goblets available in different capacities and sizes, for example, bigger goblets hold 360 medium straws, small goblets 100 straws and even smaller 25 straws. Polythene goblets are suitable than aluminium material (Fig 160).

After freezing the semen can be stored in big insulated thermos flasks or in special storing chambers. It is important that the temperature in these is kept below  $-75^{\circ}\text{C}$ . With semen kept at  $-79^{\circ}\text{C}$  it has been possible to obtain good fertility after more than five years storage but in general the fertilizing capacity of deep frozen semen will drop progressively. Stewart (1964) reported that semen stored in dry ice bath for more than nine years showed a drop in non-return rate from 66.5% to 58.0%. Mixner and Wiggin (1964) using frozen semen stored up to 8 years, reported that the fertility level was approximately the

same for semen stored for a period of two years as compared to fresh semen. The fertility of semen stored for four and eight years was lower compared to that of fresh semen and frozen semen stored for shorter periods. It is possible that a further reduction of the temperature will lengthen the storing time.

Liquid nitrogen and liquid air have been extensively used, especially USA operates on a 100 per cent frozen semen basis and deliver the semen all over the country. The semen is collected and processed at one centre and delivered in liquid nitrogen by trucks serving certain routes.

### Dispatch of frozen Semen

Different types of storage containers as well as dispatch containers are available in the market. The firms dealing with these containers are Union Carbide (U.S.A.), Minnesota Valley Engineering (U.S.A.), L'Air Liquide (France), British Oxygen Limited (U.K.). The storage containers best suited for bigger organisations are LR 750, 320 and LR 180 and freezer cum storage container LR 250 or LR 320. Dispatch containers LD 30, LNR 25, 35, XR 16 and for field use CPV 5, 10 or CD 2 litres or AL 3. Under Indian conditions Daaps (New Delhi) with collaboration of Minitub, West Germany and Tyvor Engineering Company (Madras), in collaboration with I.M.V. France are providing storage containers as 7, 20, 50, 100 and 200, and despatch containers as 7, 20, 50, and 100 litres capacity.

The storage and distribution of deep frozen semen is generally followed in the following pattern: At sub centres, one 50 litre liquid nitrogen storage con-

tainer is supplied along with containers 3 to 7 litre capacity for holding small number of straws for buyer to buyer service for insemination. It is essential to check the level of liquid nitrogen with a wooden ruler in the liquid nitrogen container and replacement should be effected to maintain the optimum level. In majority of the containers the  $LN_2$  will have to be replenished between 10-15 days interval.

### Thawing of frozen semen

Thawing of the frozen semen should be done immediately before use. There are probably two important factors for thawing the frozen semen. The first point is that the melting of the frozen semen should go as quick as possible to prevent recrystallization of the water into bigger crystals. On the other hand, once the semen is liquid it should not be warmed up too much as this could mean a sudden drop of temperature again at the time of insemination.

Cassou (1970) recommended thawing frozen semen in straws at  $+34^\circ C$ . for 15 seconds in water kept in a thermos flask. The straws should be quickly fitted to the inseminating gun previously warmed to avoid cold shock. Following steps should be taken while thawing the semen.

1. The straw should be removed with the forceps from the flask.
2. The straw should be shaken vigorously once or twice to remove liquid nitrogen from the cotton plug.
3. The straw should be placed in warm water at  $+34^\circ C$ . in thermos flask.

4. Dry the straw with a tissue paper; also wipe the scissor.
5. Warm the chamber of the insemination gun by rubbing vigorously.
6. Hold the straw vertically with the cotton plug or ball downwards.
7. Cut the straw at right angles to remove the powder plug or sealed plug through the air space.
8. Withdraw the piston of the syringe.
9. Place the straw in the warmed up chamber of syringe.
10. Take the sheath from the container and fix the sheath over the straw to ensure the firm union between the straw and sheath.

Aamdal and Anderson (1968) reported that if the straws were thawed at  $+75^\circ C$  for 12 seconds, the recovery rates were higher than thawing at  $+35^\circ C$ . Almquist and Wiggins (1973) used two thawing temperatures of  $+5^\circ C$  and  $+75^\circ C$  and found 10-12% higher recovery rates in straws thawed at  $+75^\circ C$  to 12 seconds. Rodriguez *et al* (1975) reported that the post thaw motility improved as the thawing temperature was increased from  $+5^\circ C$  to  $+55^\circ C$  ( $P < 0.01$ ). Further increase in thawing temperature to  $+90^\circ C$  did not enhance the survival, but for all practical purposes the thawing temperature of  $+35^\circ C$  for 15 seconds is recommended, since if inseminator by mistake is not particular about thawing time the semen will get unnecessarily warmed up and the motility will be reduced.

## STANDARD METHODS OF DEEP FREEZING

### A. Ampoule freezing method

1. A dilutor is prepared of three parts of 2.9% sodium citrate solution ( $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7 \cdot 2\text{H}_2\text{O}$ ) and one part of egg yolk, 1,000  $\mu$ g of streptomycin and 1,000 I.U. of penicillin per ml. is added. One half of the dilutor is used for the primary dilution of semen (Fraction I) and to the other half is added to 14% glycerol (Fraction II).

2. After collection, the semen is immediately examined microscopically on a warm stage. If the quality of semen is satisfactory, it is diluted at  $+30$ – $32^\circ\text{C}$  with fraction I of the dilutor. The diluted semen is placed in a water bath together with fraction II of the dilutor and put in the refrigerator. When both fractions have cooled to  $+5^\circ\text{C}$  after 3–4 hours, an equal volume of the glycerol containing fraction II is added to the diluted semen in 3–4 portions with intervals of about 10 minutes. The diluted semen now contains 7% glycerol and 20–22% egg yolk. The final dilution can be about 1:20.

3 The diluted semen is left in the refrigerator at  $+5^\circ\text{C}$  for 6–18 hours to allow the glycerol to equilibrate with the sperms.

4. After equilibration, the diluted semen is divided in single doses in small test tubes (1.8–2 ml.). The tubes are sealed by rubber stoppers. The identity of the semen is marked on the tubes in advance with glass writing ink. The tubes are filled and sealed partially immersed in a ice water-

bath in order to keep the temperature at  $+5^\circ\text{C}$ .

### FREEZING PROCEDURE

The freezing is carried out in a thermos jar half filled with alcohol which in the beginning has a temperature of  $+5^\circ\text{C}$ . The temperature is then lowered at a rate of  $1^\circ\text{C}$  per minute from  $+5^\circ\text{C}$  to  $-6^\circ\text{C}$  by adding small pieces of solid carbon dioxide. From  $-6^\circ\text{C}$  to  $-12^\circ\text{C}$  the temperature is lowered in one minute and from  $-12^\circ\text{C}$  to  $-30^\circ\text{C}$  the cooling rate is  $4^\circ\text{C}$  per minute. Finally the temperature is dropped from  $-30^\circ\text{C}$  to  $-79^\circ\text{C}$  rapidly by adding large quantities of solid carbon dioxide.

### STORAGE

The tubes with frozen semen can be stored in a mixture of alcohol and dry ice in thermos jars insulated by expanded styrene plastic. If liquid nitrogen is used as a method for rapid freezing and storing medium Linde container in size from 7 to 85 litres are most suitable for storage and transport.

### INSEMINATION

The tubes with frozen semen are carried to the farm in small liquid nitrogen container. Immediately before insemination, the frozen semen is thawed by immersing the tubes in water at room temperature.

### B. Pellet freezing method

1. Prepare a dilutor of four parts of a sugar solution (7.5% glucose or 10.5% lactose) and one part of egg yolk. Add to this 7% glycerol.

2. After collection and evaluation of the semen, it is diluted 1 to 2 or 1 to 3 with the dilutor at  $+30^\circ\text{C}$  to  $+32^\circ\text{C}$ .

3. The diluted semen is cooled to  $+5^{\circ}\text{C}$  in 2-3 hours and is kept at this temperature until freezing.

4. Four to five hours after collection, the diluted semen is frozen by placing small drops (0.05 to 0.2 ml) by use of a micro-syringe directly on the cavities specially made on the blocks of dry ice (Fig. 161). After the drop-lets have remained on dry ice for 2-10 minutes they are transferred to small glass tubes kept in cooled dry ice.

The glass tubes can be stored in a mixture of alcohol and dry ice or in liquid nitrogen or liquid air.

5. The pellet containing 0.2 ml. frozen semen is thawed in 0.8 ml. of a 2.9% sodium citrate solution at room temperature. The diluted semen should be used as soon as possible after thawing.

### C. Straw (Payette) freezing method

#### 1. PREPARATION OF THE DILUTOR

The dilutor Laiciphos 123 must be prepared at least one hour before use. One bag of Laiciphos gives 550 ml. and the preparation is made in the follow

mination easier but also it does not affect fertility. Antibiotics should not be added to the dilutor, as this is already contained in the commercial dilutor. A final dilution to 50 million/ml. after adding the glycerol fraction, which in the medium of payettes of 0.5 ml. giving on insemination dose of 25 million spermatozoa should be made.

#### 2. COOLING

Thermal shocks must be carefully avoided during the cooling process. The most reliable method is to use a programme cooler thermostatically controlled. If such instruments are not available, it is recommended to use one of the two following methods.

fraction. The technique must be timed for the special conditions of the individual laboratory.

### 3. PREPARATION OF THE GLYCEROL FRACTION

The same dilutor is used for both the semen and the glycerol fraction. Usually the glycerol contents of the latter is 10% but better fertility may be obtained for certain bulls with other glycerol percentages. As the semen and the glycerol fractions are equal in volume, the final proportion will be 5%.

The necessary quantity of glycerol (126 specific gravity) is heated on a water bath to 55-60°C (to increase its fluidity) and is then mixed with the dilutor, which should have a temperature of +40°C. After vigorous shaking the temperature is lowered to +4°C. A piston burette is very suitable for measuring glycerol.

The glycerol fraction should not remain standing for any length of time, as there is a tendency of stratification. It can be placed on an oscillating table awaiting the addition.

### 4. ADDITION OF THE GLYCEROL FRACTION

The glycerol fraction can be added to the semen in portions at 10-15 minutes intervals, where the volume of the portions are gradually increased (e.g. 10%-20%-30%-40%), but it is considered to be better to add it drop by drop while oscillating or stirring. The size of the flasks must be big enough, so that they are not more than half filled after adding the glycerol fraction. A 250 ml. flask should in no case be used for more than 160 ml. glycerolated semen and a 500 ml. flasks for maximum 350 ml.

The speed of adding the drops should be regulated so that the glycerolization is completed in half an hour or three quarters of an hour for bigger volumes.

### 5. EQUILIBRATION TIME

Formerly an equilibration time of 15-18 hours was recommended. The semen was collected in the afternoon and frozen the following days. Now morning collection is used, with an equilibration time of 7-8 hours.

### 6. PRINTING

The following information in the given order ought to be printed on the payettes:

Code number of the AI units, code number of the bull, operation number of the freezing, year number, name of bull and herd book number.

The colour of the ink must be chosen with regard to the colour of the payette. Black ink can be used for most of the payettes as they become much lighter when filled, for the darkest payettes silver ink seem to be best.

Immediately after printing the straws should go for drying of the ink to the special cabinet with a temperature not exceeding 45°C.

### 7. FIXING IN CLIPS, STERILIZING

When the print is dry, the payettes are fixed 15×15 in fixing clips and are put into a refrigerator or refrigerated cabinet.

### 8. FILLING

This and the following steps are made in a cool room or in refrigerated cabinet. After the glycerolization has been finished and the payettes have been prepared as mentioned above, a quantity of the semen is poured into a low beaker or crystallization jar. The semen must be stir-

red if it has been standing for a while. A clip with payettes is applied to a filling nozzle, connected with a vacuum pump. The free hands of the payettes are dipped into the semen, the back opening of the filling nozzle is pressed by the tip of finger, and the semen is sucked up into the payettes. When all are filled the air passage through the back opening is left free again and the payettes are taken away from the filling nozzle.

The clip is grasped between thumb and fore finger and with a sudden jerk (some training is necessary) the extremities of the straws are emptied (17 mm. is ideal). The ends are dried on a sterile filter paper.

#### 9. PLUGGING

A few glass plates, about 20×30 cm. are needed for the plugging. The edges should be rounded to avoid injuries.

A layer of plastic powder (appropriate colour) is spread to a thickness of 1 cm. on a glass plate, the spreading is easily made with a frying slicer-clip. After clips are taken the ends of the payettes are dried against a filter paper and then pressed into plastic powder three or four times, so that the powder fills the outer part, about 7 mm. The payettes are loosened from the clip and dropped into a water bath of +4°C where they are left for the better part of the equilibration time or at least for two hours. The plugs are fixed and most of the excess powder is detached into the water

part of it is folded over the payettes, which are now dried by rolling under cautious pressure. The procedure is made once more with a dry towel. All payettes must roll along and they should not roll over each other, otherwise the drying becomes less effective and there can be slight damage.

When the payettes are dry, they are held vertically, so air bubbles go to the tampon end. They are turned and struck quickly so the air bubble is driven to the plug end. Finally the payettes are placed upon freezing ramps, and once more it should be observed that all air bubbles are just up to the plug. The spreading on the ramps is easy to perform if the payettes are quite dry but difficulty may be found if they are wet and sticky.

#### 11. PREPARATION OF FREEZING

The freezing is performed in a container with large opening i.e. Linde 250, MVE A 9000 or RCB 100 F Containers with an opening of medium size can be used but then the work is more difficult and more time consuming.



be used. If MVE A9000 is used, the nitrogen level must be 1 cm. under the grill. After this has been checked, the top cover is put on for at least 5 minutes for stabilization of the temperature. A plywood cover is used.

## 12. FREEZING

The freezing is simply made by placing the ramps gently on the grill. The payetts are then 4 cm. above the nitrogen level in Linde 250 and RCB 400 and 5 cm. in MVE A9000. The plywood cover is put on immediately avoiding turbulence in the container.

The temperature of the payetts is lowered quickly and reaches  $-100^{\circ}\text{C}$  in 5-6 minutes. After 7 minutes the cover is taken away and the payettes are gathered a handful at a time and plunged into the funnel. When all payettes are put into the liquid nitrogen, the ramps are taken away and the goblet in the pit is placed in its definite place, if another freezing is to be made.

## 13. HANDLING OF FROZEN PAYETTES

After being frozen and plunged into the goblet the payettes ought to be stored constantly under the surface of liquid nitrogen.

Immediately after freezing, the payettes are brought into standard goblets and they are then kept in such goblets all the time. The goblets can be divided by a partition wall, splitting cross, polygonal or hexagonal casing, so different operations or straws of different bulls can be stored in one goblet.

Transfer of payettes from one container to the other should always be made in goblets, filled with liquid nitrogen. The transfer ought to be prepared, so it can be made quickly. This is of special importance if a goblet is quite filled with

payettes, as it's contents of liquid nitrogen is only about 60 ml. i.e. 18% of the total volume.

## 14. STORAGE OF FROZEN PAYETTES

The standard goblet is said to hold 370 medium payettes, but as a rule one should not have more than 340-350 in each.

In the laboratory the following categories of storage can be distinguished.

- (a) Storage for distribution.
- (b) Buffer storage.
- (c) Long time storage.

## Conception rates in Cross-breeds

Chinnaiya *et al* (1974) worked on deep freezing of semen of Holstein, Jersey and Red Dane breeds by using glass ampoules stored in liquid nitrogen containers. They obtained the average conception rate of 46.9%, 50.8% and 50.0% respectively. Mathew (1974) observed the semen characteristics of cross-breeds and pure-breeds (Brown Swiss) bulls at Indo Swiss Project Kerala. The semen was frozen in 0.5 ml. straw by rapid horizontal vapour freezing technique using liquid nitrogen. The general tendency was for increase in semen volume, sperm concentration and freezability with the increase rate of exotic inheritance. Apparently, there was a difference between 50% cross-bred and pure-bred bulls in their capacity to produce quality semen. 75% and 62.5% cross-breeds appeared to be nearer to the European pure breeds. The overall conception rate varied from 35 to 45%.

Tandon (1975) analysed the data of all India cross-breeding projects and concluded that the overall conception rate both with liquid as well as frozen semen of exotic bulls ranged between 30 to 33%. Kaikini (1976) recorded an over-

all conception rate of 54.31% using semen in 116 local cows for cross breeding requiring 2.95 inseminations per conception. Vasanth and Nagaraj (1978) reported the overall pregnancy rate from 49% to 57% based on 17,000 doses issued to 50 different organisations located all over India.

### DEEP FREEZING OF BUFFALO-BULL SEMEN

Deep freezing of buffalo bull semen is only taken up during the recent years. This is on account of the fact that milch buffaloes are usually located in the milk pockets where sufficient number of high grade bulls are not available for natural service. Moreover, the breeding is seasonal. It is also a matter for consideration that the milk yield in buffaloes has not exceeded above 10-12 litres even with best possible procedures of selective breeding. This is in contrast to breeding of milch cattle where levels of production are even as high as 30 to 40 litres. Low keeping quality of buffalo

other methods. Further Pavithran *et al* (1972) reported milk egg yolk sodium citrate lactose and glycerol diluent for freezing of buffalo semen. Sahni and Roy (1972) reported the effect of freezing at  $-79^{\circ}\text{C}$  on the percentage of motile sperms in EYC dilutor as 12% and milk dilutor as 13%. The semen of only one buffalo bull out of 10, could withstand the deep freezing process. Roy and Ansari (1973) observed highly significant variation between buffalo bulls in the freezing characteristics of semen. Roy and Bhat (1973) also found that EYC frozen buffalo semen was significantly superior to other extenders i.e. EYC-glucose and egg yolk glucose bicarbonate, on consideration of post freezing per cent of motile spermatozoa. Bandyopadhyay *et al* (1971) observed that EYC dilutor was significantly superior to milk dilutors.

couraging i.e. 25.5% for deep frozen (145-inseminations) as against 24.04% for liquid semen (111-inseminations).

### Dilutors for freezing buffalo semen

Some common diluents which could be used for freezing buffalo semen as well as cow bull semen are as follows (Bhosrekar, 1977):—

1. Diluent—I
 

Sodium citrate	2.6% W/V
Fructose	2.0% W/V
Egg yolk	10.0% V/V
Penicillin G Sodium	500 i.u./ml.
Streptomycin	500 $\mu$ g/ml.

 Diluent—II  
 All the constituents of diluent I plus 16% glycerol.
2. Diluent—I
 

Sodium Bicarbonate	1.3%	1 part
Glucose 5%		4 parts
Egg yolk		1 part

 Diluent—II  
 All the constituents of diluent-I plus 14% glycerol.
3. Diluent—I
 

Skim milk heated to 92°C for 10 minutes and Cooled	80 parts
Egg yolk	20 parts

 Diluent—II  
 All the constituents of diluent-I plus 20% glycerol.

### 4. Citric Acid Whey (CAW)

Ready formula mix in 100 ml. distilled water, filter through cotton in another clean beaker, adjust the pH of filtrate to 6.8 exactly with freshly prepared 10% NaOH, add penicillin and streptomycin at 1000 i.u. per ml. and 1.5 mcg. per ml. respectively. Divide in equal volumes as Diluent I and II. To diluent I add 3% glycerol and diluent II and 11% glycerol.

5. Laiciphos—123 prescribed with 10% egg yolk. One bag of laiciphos gives 550 ml. of the dilutor.

### 6. Standard solution

(a) Tris	3.87 g.
(b) Fructose	1.27 g.
(c) Citric acid	1.73 g.
(d) Dist. water	99.13 ml.
Standard solution	74.0 ml.
Egg yolk	20.0 ml.
Glycerol	6.0 ml.
Dihydrostreptomycin	100 mg.
pH adjusted to 6.8 with citric acid or NaOH.	

### 7. Diluent—I

Sodium citrate 2.9%	75 ml.
Egg yolk	25 ml.
Fructose	2.5 g.
Diluent—II	
Sodium citrate 2.9%	75 ml.
Egg yolk	25 ml.
Glycerol	14 ml.

### Deep freezing of Ram and Buck Semen

Emmens and Blackshaw (1950) reported for the first time the possibility to revive ram spermatozoa after freezing to  $-79^{\circ}\text{C}$  with glycerol concentration of 7.5% and with the addition of various sugars, particularly pentose. Galkin (1954), Blackshaw (1955), Morozov (1957), Lopyrin and Loginova (1958), Hill *et al* (1958), Feredean and Bragaru (1963) and Morozov (1964) did not get satisfactory results in freezing of ram semen. The technique of freezing semen rapidly by pelleting directly on dry ice was first demonstrated successfully by Nagase and Niwa (1963, 1964) in bull spermatozoa. This method was subsequently adopted for freezing of ram spermatozoa (Platov, 1965, 1966 and Platov and Sevcova, 1966). Salamon (1968) reported that there was no significant differences in revival rates of ram spermatozoa after adopting pellet method on dry

ice or freezing in ampoules at slow rate and both the methods were superior to freezing in synthetic straws. Fraser (1968) reviewed the progress of freezing ram semen and opined that freezing by pellet method is a standard one for ram spermatozoa.

Smith and Polge (1950) reported that upto 90% revival of bull spermatozoa and complete revival of goat spermatozoa was possible after relatively slow freezing in 15% glycerol. In spite of this report on the successful recovery of motile spermatozoa after freezing to  $-79^{\circ}\text{C}$ , there has been no report on satisfactory breeding trials in goats. Barker (1957) reported the results as "too low to be of practical value". Results reported on deep freezing of goat spermatozoa were not encouraging (Tierzucht, 1960; Liess and Ostrowski, 1960; Weiss Flog, 1961; Kalev and Venkov, 1961). However, Bonfert (1969) obtained satisfactory recovery and conception rates after freezing of goat semen. Hahn (1972) obtained 69% kidding after using frozen pelleted semen.

Hukeri *et al* (1977) tried egg yolk tris-glucose citric acid-glycerol dilutor to freeze ram semen by pelleting on dry ice and storing in liquid nitrogen which gave 47.65% revival of spermatozoa. They found egg yolk-tris-fructose citric acid-glycerol dilutor comparatively better to freeze buck semen by pellet method which gave 49.53% revival of spermatozoa. Further in these dilutors Nimkar (1977) reported in details deep freezing of ram and buck semen by pellet method and assessed the keeping quality by studying seminal attributes viz. motility score, percentage motile, live and abnormal percentage at different temperatures viz.  $+30^{\circ}\text{C}$ ,  $+5^{\circ}\text{C}$  and  $-196^{\circ}\text{C}$  and between 'O' hour and 7, 14, 21 and 28 days of preservation in liquid nitrogen. He observed the mean progressive motile percentage of ram and buck spermatozoa at 28 days preservation as  $41.48 \pm 2.64\%$  and  $44.07 \pm 1.45\%$  respectively. Based on these studies the following dilutor is recommended to freeze ram and buck semen by pellet method.

Dilutor for Ram Semen as per Miller (1975)			Dilutor for Buck semen As per Hahn (1972)	
Tris	360 mM	4.356 g.	Tris	6.056 g.
Glucose	33.3 mM	0.6 g.	Fructose	2.50 g.
Citric acid	113.7 mM	2.388 g.	Citric acid	3.40 g.
Add. Distilled water		76.0 ml.	Distilled water	181.0 ml.
Glycerol		6.0 ml.	Glycerol	16.0 ml.
Egg yolk		18.0 ml.	Egg yolk	50.0 ml.
		100.0 ml.		

(pH 6.98-7.00)

#### Deep freezing of Poultry Semen

It is considered that frozen semen will be of considerable importance in the years to come in poultry production programmes in various countries of the

world. Shaffner (1942) recorded his experiments on quick freezing of semen at  $-76^{\circ}\text{C}$  and storage for 14 months resulting in some fertility but no hatchability. Smith and Polge

(1951) found that by adding 15% glycerol before freezing at  $-79^{\circ}\text{C}$ , the sperms revived to a high degree of motility but gave no fertility. Polge (1951) followed the same technique but removed the glycerol by dialysis with ringer solution and obtained 54% fertility during the week following insemination. Allen and Bobr (1955) showed that fertility from semen glycerol mixtures can be obtained by inseminating directly into the uterus.

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**PART—V**

**SPECIAL CHAPTERS**

# Chapter 49

## The Mammalian Egg and Transplantation of Fertilized Ova

The mammalian ovary contains enough oocytes to produce many times the normal number of eggs if properly stimulated. Bovine ovaries, for example, contain some 133,000 (range = 0 to 700,000) primordial follicles (Erickson, 1966). The number seems to remain stable for a given cow until she is 4 years of age and then begins to decline. Artificial insemination has been an important method for the widespread distribution of desirable genes by way of the sperm. Similar genetical improvement in farm animals via the egg has been greatly hampered by the inability to utilize the potential ova of a female.

However, superovulation has made it possible to transfer the ova of genetically superior animals to genetically inferior ones. Superovulation refers to the experimental increase in the number of follicles ovulated at any given time in prepuberal and adult animals. Gonadotrophins have been used to induce superovulation before puberty and during season and lactational anestrus in sheep, cattle (reviewed by Hammond, 1961), and man (Gerzoff, 1962).

Studies on superovulation provide basic information about ovarian function, e.g. about the maturation of follicular oocytes and its hormonal control. Mild superovulation is used to increase litter size in monotocous species such as cattle and sheep. The transfer of embryos has both scientific and practical advantages. It allows critical experimental approaches to problems in the physiology and biochemistry of reproduction, developmental genetics, cytology, animal breeding, immunology, and evolution. For example, the technique can be used to study capacitation of sperm, transfer of ovarian eggs, interaction between the embryo and the uterus (Chang and Pickworth, 1969), immunological relationships between the conceptus and the fetus, interspecific egg transfer (Chang, 1966), ectopic egg transfer (Kirby, 1965), and experimental manipulation of the embryo (McLennan, 1969).

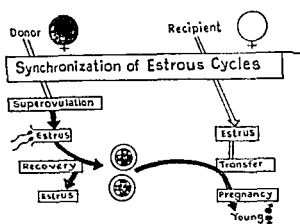


Fig 162. Schematic diagram-steps involved in egg transfer in cattle, sheep and swine

for a given dam, and provide a large number of full sibs of the same age. The technique could also be utilized to obtain superior slaughter animals (beef calves) from dairy cows. It would also permit international transfer of animals at low cost; for example, successful long-distance transport of sheep embryos has been accomplished in the reproductive tract of a rabbit (Adams *et al*, 1961).

## 1. SUPEROVULATION IN IMMATURE ANIMALS

The ovaries of prepubertal farm mammals contain 100,000 to 300,000 oocytes. The number of ova which they ovulate during an average lifetime is certainly a very small percentage of the ovarian potential. Follicles as large as 5 mm. in diameter have been observed on the ovaries of calves at one week of age (Marden, 1953). Ovaries of slaughterhouse calves, 14 weeks of age possess an average of 10 vesicular follicles larger than 1 mm. in diameter (Casida *et al*, 1935). Growing follicles rapidly increase in number in calves between 50 and 80 days of age (Erickson, 1966).

Prepubertal animals respond to superovulatory conditions as soon as the follicle develops an antrum. From birth to

puberty the sensitivity to gonadotrophins increases gradually and at rates which are species and probably breed specific. The ovaries of immature sheep, for example, can be stimulated at 16 weeks of age (Mansour, 1959), and those of rabbits at 16 to 20 weeks (Kennelly and Foote, 1965), depending upon the breed and plane of nutrition. Immaturity and poor nutritive conditions seem to impair follicular growth and ovulation in response to gonadotrophins. The development of eggs into normal young indicates that eggs obtained from immature donors are functionally equivalent to those of adults.

## A. Cattle

Follicular growth has been stimulated in the calf by FSH from different sources. Ovulation may be induced by endogenous or exogenous LH (Black *et al*, 1953). This may indicate that in the normal female the LH threshold in ovarian tissue is high. LH release is low, or the pituitary reserve of endogenous LH is small. These treatments produce inconsistent degrees of follicle proliferation and ovulation. The percentage of calves ovulating after gonadotrophin treatment ranges from 10 to 85 per cent (Marden, 1952; Avery *et al*, 1962a; Jainudeen *et al*, 1966a). The variation in ovary size, number of follicles, and number of ovulations among gonadotrophin-treated calves is very large, whether the calves are 3 weeks of age or 6 to 8 months old.

Stimulation of estrous cycles in calves has been attempted by stimulating corpora lutea formation with gonadotrophins (Howe *et al*, 1962), or by injecting progesterone before gonadotrophin administration to stimulate luteinization (Avery *et al*, 1962a; Howe *et al*, 1961).

Table 70  
SUMMARY OF SOME OF THE HORMONAL TREATMENTS USED IN  
SUPEROVULATION IN ADULT FARM ANIMALS (HAFEZ, 1969)

Species	Hormonal Treatment	Reference
Cattle	swine pituitary extract, following expression of C.L. series of progesterone injections	Avery et al (1962a, b)
	2000-3000 I U of PMSG on day 16 followed 4 days later with 2000 I U of HCG	Hafez & Sugie (1961) Hafez et al (1963, 1963)
	PMSG following expression of C.L. or after last of a series of injections or feeding of a progestogen	Jamudeen & Hafez (1966)
Sheep	600-1000 I U of PMSG (according to body weight) on day 12 or 13	Hunter et al (1955) Rouson & Moore (1966a)
	5 subcutaneous injections of 12 mg. each of horse anterior pituitary extract following progesterone treatment	Shelton & Moore (1967)
Pig	750-1500 I U PMSG at day 15 or 16 + 500 I U HCG at onset of estrus	Hunter (1964)
	1200 I U PMSG at day 15 or on day of weaning	Phillippo (1968)
Rabbit	3 injections of 50 I U of PMSG, intramuscularly on 3 successive days + 30 I U of HCG intravenously	Hafez (1961a, c)
	twice daily injection of 0.5 mg FSH for 3 days + 1 mg. LH/kg. body-weight	Varian et al (1967)
	twice daily injection of 0.3 mg. FSH subcutaneously + 2.5 mg. pituitary LH intravenously	Mauer et al (1968)

sembled that of the control gonad; however, if the corpus luteum was not entirely removed the number of follicles which developed was appreciably lower.

#### SHEEP

PMSG given on the 12th or 13th day of the cycle causes superovulation (Fig. 163). As the dose level increases from 700 to 1500 I U, the number of eggs recovered increases from 3 to 9

(Iverill, 1958). Equine anterior pituitary extract is also a potent superovulator, the number of ovulations increasing from 1 to 9 with daily doses of 60 to 135 mg. begun on day 12 after estrus (Moore and Shelton, 1964). Superovulation can also be induced with FSH injected after progestin treatment to control the time of estrus (Robinson 1961, 1965).

## Pig

Superovulation can be induced in the gilt by the injection of PMSG on days 15-17 of the estrous cycle. Treatment on day 15 is more likely to cause superovulation (Phillippo, 1968). Cystic follicles and anestrus subsequently develop, apparently from PMSG treatment *per se*, rather than the day on which treatment is given. One subcutaneous injection of 1250 I U of PMSG is also effective when administered 24 hr. after the last day of methallibure treatment (Dziuk, 1969).

Induction of ovulation in the luteal phase of the estrous cycle, unlike that in the follicular phase, requires exogenous LH. In the rat, progesterone decreases the ovulatory response to exogenous gonadotrophins (Callantine *et al*, 1964; Callantine and Humphrey, 1965) by blocking the release of pituitary hormones. The high level of circulating progesterone during the luteal phase of the estrous cycle (Gomes *et al*, 1965) may have a similar effect in the pig. No information is yet available on the timing of ovulation in PMSG-treated pigs.

## RABBITS

Repeated superovulation and *in vivo* collection of eggs was performed by Maurer *et al* (1968). Injections of FSH-LH at 16, 32, 48, and 56 weeks resulted in 47, 35, 25, and 18 ovulation points respectively, and an 83% fertilization rate. Superovulation can also be induced in the pregnant rabbit. The eggs are apparently normal as shown by the presence of the first polar body and the second maturation spindle. They are physiologically normal because they can be fertilized either *in vitro* or after transfer to the oviducts of mated rabbits (Chang, 1961).

Superovulation in farm animals has been reviewed by (Donker 1952), (Laming and Rowson, 1952), (Willett, 1953), (Nichols, 1956), (Dziuk *et al*, 1958), (Hafez, 1958), and (Hammond, 1961).

## B. Ovulation and Estrus

Ovulation is usually induced with an intravenous injection of LH at estrus. However, there is no experimental proof that this procedure results in a larger number of ovulations or a higher fertilization rate. In the presence of a corpus luteum, however, follicles developed with PMSG or FSH do not generally ovulate spontaneously. It is possible that progesterone from the corpus luteum blocks the release of endogenous LH.

The rupture of the first follicle starts about 24 hr. after HCG injection. The ovulation of subsequent follicles may continue, at least in prepuberal calves, for the next 48 hr. The biological half life for blood-borne PMSG is some 36 hr. in the rabbit (Lamond, 1960) and 48 hr. in the rat (Green, 1961). The superovulatory response depends on the amount of hormone available for follicular growth and the time available for its action.

The physiological mechanisms which cause ovulation are quite independent of those which cause estrus. In fact, both phenomena can be separated by appropriate brain lesions. Gonadotrophin treated cows may be due to changes in timing or estrogen secretion under an altered FSH: LH balance, or even to an adrenal corticoid antagonism with estrus, but not ovulation.

## VARIABILITY

There are quantitative and qualitative differences in the responses of animals to

crude and purified PMSG. Doses assayed as equipotent in the rat do not produce equal responses in sheep and cattle. Purified PMSG yields less follicular growth and a lower ovulation rate than pregnant urine (PU) (Rowson, 1951). This may be due to less synergistic LH activity during purification or to differences in rate of absorption or excretion.

Some animals show little follicular growth even after large doses of PMSG, whereas others exhibit great superovulatory responses after small doses. Modifications of the dosage and interval between treatment with PMSG and HCG have not yielded consistent results.

The superovulatory responses of mature animals vary with the season of year, breed, live weight, stage of the estrous cycle at injection, individual variations in cycle length, age, genetic constitution, the amount of hormone administered previously, the post-partum interval, and plane of nutrition. For example, the superovulatory response of the rat is enhanced by increasing the post partum interval. The number of ovulated eggs after gonadotrophin injections varies with the potency of the hormone preparation, the FSH:LH ratio, the frequency of successive injections, and the dosage and mode of administration of hormones.

Dividing of PMSG dose into six parts administered over 24 hr. nearly halved the variability of the response in one strain of mice and reduced the variance sixfold in another (McLaren, 1967). The degree to which division of the dose affects the superovulatory response depends on the relationship between the biological half life of the hormone and the interval between successive doses of the hormone. Endogenous FSH is pre-

sumably released continuously from the pituitary, at least during some periods of the estrous cycle. Information about the biological half-life of exogenous FSH is scanty and conflicting.

#### REFRACTORINESS

When immature rabbits are treated every 28 days with horse anterior pituitary extract, superovulation does not occur (Adams, 1953). There is no decline in the superovulatory response of rabbits injected twice with 25 mg of LH (Foote *et al*, 1963.) However, the superovulatory response falls after four successive injections (Maurer *et al*, 1968) and ceases after six injections (Adams, 1961). Refractoriness to repeated injections also occurs in cattle (Willett *et al*, 1953), sheep (Palsson, 1962), and mice (Edwards and Fowler, 1958; Lin and Bailey, 1965).

This refractory state has been attributed to the formation of antibodies in the pseudo- and euglobulin fractions of the blood in treated animals.

#### C. Fertilization and Egg Recovery

In general, fertilization rate is lower following superovulation in farm animals than in laboratory animals. Low fertilization rate is partly due to lack of sperm transport after vaginal or cervical insemination. It is also possible that capacitation of sperm no longer occurs in the reproductive tract of gonadotrophin-treated females. Further studies are needed on sperm transport, cervical function, and the properties of cervical mucus in gonadotrophin-treated animals.

Overstimulation of the ovary causes reduced rates of egg recovery. This is partly due to the effect of endogenous estrogen production on the rate of egg transport and partially to eggs being

trapped in follicles. Rapid oviductal transport under the influence of several corpora lutea would cause the eggs to reach the uterus before they could be recovered. Ovary size also influences the rate of egg recovery. The recovery rates in cattle are higher from small ovaries containing 10 or less follicles than larger ones, possibly because the fimbriae of gonadotrophin-treated animals do not enlarge as much as they do during normal ovulation.

### EGG COLLECTION

Eggs are collected from the donors using *in vitro* or *in vivo* techniques (Fig. 164). In *in vitro* methods the animals are autopsied, and the oviducts are removed and placed in sterilized petri dishes. The eggs are flushed from the oviducts with a sterile physiological saline-serum solution. The eggs are collected using a glass pipette, under a stereoscopic binocular microscope and transferred through two sterile solutions

of saline to decrease any possible contamination, counted (X15) and carefully examined (X40 to X100) for abnormalities.

The rate of transport of the egg within the oviduct varies slightly with the species, being slightly faster in the pig than in cattle and sheep, probably as a result of multiple corpora lutea and the relatively higher levels of progesterone. Superovulation in cattle tends to accelerate the rate of oviductal eggs.

Fire-polished glass pipettes of a proper diameter (1 to 2 mm.) are used to handle the eggs. During manipulation as little exposure to visible light as possible is desirable. For prolonged exposure, red light is preferred; short exposure to ultraviolet light may inhibit cleavage of rabbit eggs (Daniel, 1964). All equipment used for handling the eggs should be sterilized and held at 30° to 37° C either on a warm plate or in an incubator. In the interval between recovery and storage, the eggs could be stored in the culture cabinet at room temperature.

Holding sheep eggs for 12-60 minutes at room temperature before cooling at 10°C does not affect their survival when stored for 5 days *in vitro* or their subsequent development *in vivo* (Kardymowicz *et al*, 1966); exposure to daylight for 1 hr. was likewise not detrimental.

### EGG SELECTION

The integrity of an egg is important for its survival both *in vivo* and *in vitro*. Microscopically visible as well as non-visible defects may be present; such eggs may or may not implant (Hafez, 1962a). Moreover, defective eggs that do implant may or may not survive during subsequent embryonic and fetal life.

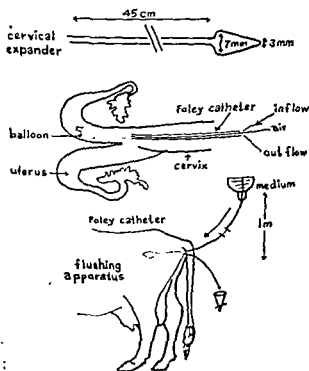


Fig. 164. Non-surgical recovery of bovine ova.

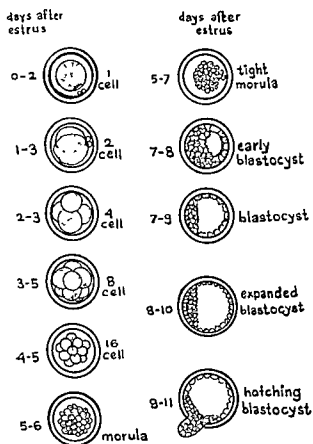


Fig 165 Morphologically normal bovine embryos recovered at various stages of development

Structural abnormalities in the egg may result from cytological, genetic, environmental, pathologic or artifactual factors; the former three may contribute to the variability in successful egg storage. Such anomalies that have been described (Hafez, 1961b), include aberrations in size, shape and degree of cytoplasmic granulation or pigmentation. Morphologically abnormal eggs may have the shape of a helmet, kidney, amoeba, ovoid, or paramecium. Abnormally shaped eggs and lentil shaped ova do not develop normally, although oval-shaped ova do. Thus, eggs used for storage should be classified according to the stage of cleavage and examined under a high magnification (X40 to X100) for any malformation in the structure (Figs. 165, 166, 167).

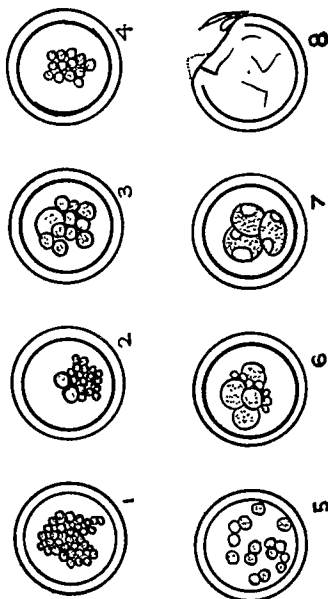


Fig. 166. Morphologically abnormal embryos.

1. Tight morula with oval zona.
2. Morula with excluded blastomeres.
3. Irregular blastomeres.
4. Morula with debris.
5. Loose blastomeres.
6. Irregular cell mass.
7. Vacuoles in cytoplasm.
8. Cracked empty zona pellucida.

### STORAGE OF EGGS

Refrigeration, low temperature freezing, freeze drying, and ultra-rapid freezing have been among the methods used to preserve blood cells, malignant cells, skin tissues, glands, and even intact lower forms of animal life (Belehradek, 1935; Parkes, 1956). Fertilized eggs of the



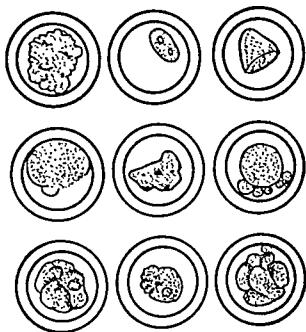


Fig. 167. Degenerating one cell ova. Not to be used for embryo transfer.

ant (Pickett, 1893), fruit fly (*Bach and Pemberton*, 1916), and chicken (*Moran*, 1925) do not survive prolonged exposure to cold. In contrast, those of *ascaris* can be stored at 5°C for a few months and still develop normally upon rewarming. Furthermore, fertilized eggs of some species do not develop unless previously cooled for a definite period (*Greeley*, 1903).

The fact that delayed implantation is fairly common in a number of mammalian species prompted investigation concerning the storage of mammalian eggs. For practical use in egg transfer experiments, it is desirable to maintain the egg *in vitro* in a more or less dormant state for various periods of time allowing accurate synchronization of egg development and endometrial differentiation. Storage of eggs also makes possible long-distance transportation of embryos (*Marden and Chang*, 1952; *Chang and Marden*, 1954; *Adams et al*, 1961; *Hunter et al*, 1962).

The eggs of several mammalian species have successfully been stored for a few days at sub-normal temperatures.

The survival rate depends on the species, the developmental stage of the egg, physical and biochemical properties of the storage media, storage temperature, the rate of cooling and rewarming the eggs and the techniques of storage and egg transfer. Fertilized eggs are more resistant to cold storage than unfertilized eggs. Unfertilized rabbit eggs recovered 2 hr. after ovulation could be kept at 0°C for 48-72 hr., or at 10°C for up to 96 hr. and still undergo fertilization after transfer (*Chang*, 1952, 1953, 1955). While fertilization seemed normal, most of the embryos degenerated before birth.

The storage medium should provide an environment in which the egg can maintain its integrity even though its metabolic processes are slowed down. To date, most storage mediums consist of salt solutions, serum, proteins and antibiotics (*cf. Paul*, 1960).

Physiological saline, containing 0.9 per cent NaCl, maintains the tonicity of eggs for short periods, but it lacks both buffering capacity and many essential organic ions. Various preparations have been used for manipulating eggs, such as Krebs solution (*Black et al*, 1951) and phosphate-buffered Ringer-Dale solution containing glucose (*Chang*, 1952). Omission of calcium, magnesium, potassium, or glucose from Krebs-Ringer bicarbonate prevents growth of mouse eggs, whereas omission of phosphate results in delayed development (*Whitten*, 1956).

Physiological solutions are used: (a) to serve as flushing fluids, while maintaining tonicity with the vitellus of the eggs; (b) to buffer the medium to and maintain it in the physiological pH range (7.2-7.6); and (c) to provide an aqueous ionic environment for cell metabolism (*Parker*, 1961).

Most of the successful egg storage media contain serum. Routine storage media contain 25 to 50% homologous serum (Chang, 1949; Gates and Runner, 1952; Hancock and Hovell, 1962; Hancock, 1963; Hunter *et al.*, 1962; Kvasnichii, 1956; Pincus, 1936, 1939; Willett *et al.*, 1953). However, occasionally the serum of other species can be used; i.e., horse, dog, guinea pig, rat and pig serum can be used to store rabbit eggs whereas, in an autoclaved centrifuge tube, the serum from man, sheep, cattle, goat and fowl contains an ovicidal factor against rabbit eggs (Chang, 1949). Fresh serum can be substituted for by: serum previously frozen at  $-20^{\circ}\text{C}$ , reconstituted freeze-dried serum, blastocyst fluid, and egg yolk citrate. Homologous serum is unsuitable medium for egg transfer in cattle but tissue culture medium 199 is highly satisfactory (Rowson *et al.*, 1969).

Rabbit blood is collected by heart puncture and allowed to clot for 30 minutes at room temperature prior to centrifugation 20 min. at 3000 rpm. The serum is decanted and recentrifuged, drawn into a 10 ml. syringe, filtered through  $0.45\ \mu$  millipore filter into an equal volume of sterile 0.9 per cent physiological saline solution and stored at  $3$  to  $4^{\circ}\text{C}$ . Furthermore, serum previously frozen for 1 month does not affect the viability of stored rabbit embryos. Eggs do not survive when stored in chicken egg white diluted with Tyrode solution (Hafez, 1965).

Gelatin has been used in storage experiments also. This medium is prepared by dissolving 14 grams per ml. of saline and autoclaving immediately after preparation to avoid any bacterial growth. After storage in 7% gelatin, the rate of implantation of rabbit eggs

was 53% after 7 days and gradually declined until it reached 3% after 14 days; the addition of antibiotics did not prolong survival time (Hafez, 1961c, 1962b). The addition of 7% gelatin to the storage medium is recommended.

A concentration of 0.02 per cent streptomycin in a culture medium containing serum prevents bacterial growth even when manipulations are carried out under antiseptic conditions. The cleavage of rabbit eggs is not affected by penicillin at concentrations up to 10 per cent, whereas streptomycin at concentrations greater than 1 per cent inhibits, but less than 1 per cent has no effect. Aceto-sulphamine and sulfamerazine also somewhat inhibit cleavage (Kurosaki *et al.*, 1954).

The survival of eggs is not affected when they are stored at low temperatures in concentrations of 7.5 mg. of streptomycin, 4 mg. chloromycetin, 6.5 mg. paromomycin, 23.9 mg. of penicillin per ml. of liquid medium. At higher concentrations, the number of survivors is either partially or completely abolished. Egg survival is neither prolonged in liquid media in the presence of antibiotics in half lethal doses, nor in the presence of lesser doses, in gelled media (Hafez, 1962b). Evidence concerning the effects of storage in physiological levels of antibiotics on subsequent embryonic survival are not available.

### Storage Containers

Four ml. glass tubes with an 8 mm. diameter inside are used. Gelatin, saline and serum are measured with sterile syringes and transferred to the tubes. When the final volume of storage medium in the tube is reached, the medium is then thoroughly mixed, with a glass pipette fitted with a rubber bulb. Care

is taken to avoid the formation of air bubbles. Filling the tubes with water will prevent loss from the storage medium by evaporation and condensation. Ten to one hundred eggs can be stored in one tube.

### Storage Temperature

Several investigations have been undertaken to study the effect of cold storage on mammalian tissues. The temperature below which the cell membrane of rat diaphragm muscle is unable to maintain its cellular ionic components is 15 to 17°C (Taylor, 1956). When mammalian eggs are stored below 20°C, they do not undergo further cleavage. Thus, the 2-day rabbit egg, after storage, is synchronized with recipients which were injected with LH two days before egg transfer.

The optimal temperature for storage has been estimated as 10°C for the rabbit (Chang, 1947, 1948a, b); 5° to 10°C for the rat (Sugawara and Takeuchi, 1962); 5°C for mouse (Sherman and Lin, 1959), and 7°C for the sheep (Averill and Rowson, 1959; Harper and Rowson, 1963). Two-celled rabbit eggs survive in rabbit serum at 0°, 5°, or 15°C for 96 to 120 hours. At 22 to 21° C they are viable for only 24, 50 and 48 hours (Chang, 1948b).

### Storage Period

The period which the egg can be stored without losing its viability and without causing a harmful effect varies with the species, stage of development of the egg, and the storage medium and temperature.

Rabbit eggs have apparently been stored for longer periods than any other mammalian eggs. Such eggs differ from

most other species in that they become covered with a mucin coat during transportation in the oviduct. Although evidence is lacking, this layer may protect the egg during manipulation and storage *in vitro*. If so, it may be possible to increase the resistance of non-coated eggs of other species by storing them in oviducts of rabbits for one day before *in vitro* storage.

Eggs of other species that have been stored include: four to twelve-celled eggs of sheep for 48 hours in heat-treated sheep serum with added streptomycin developed to term (Buttle and Hancock, 1964); sheep eggs in autologous serum, viable for periods ranging from five hours (Hancock and Howell, 1961) to a few days (Averill and Rowson, 1959). The percentage of eggs that survived storage at 10°C was 18 for eggs stored for 5 days and 63 for eggs stored for 3 days (Kardymowicz *et al*, 1966). Unfertilized mouse eggs survive upto 3½ hours in a modified Locke's solution containing 5% glycerol at -10°C (Sherman and Lin, 1958), and up to 6 hours at 0°C (Sherman and Lin, 1959).

### Storage of Eggs in the Reproductive Tract of Another Species

Eggs of one species can be transferred into the oviduct or uterus of another species, and after short term storage, may be recovered for subsequent transfer to an appropriate recipient. Little information is available concerning survival of fertilized eggs after interspecific egg transfer (Warwick and Berry, 1949; Briones and Beatty, 1954; Jernst *et al*, 1955). Fertilized eggs can survive and develop in the rabbit oviduct, but in the uterus. Rabbit eggs on the other hand cannot survive in either the ferret or

duct or uterus (Chang, 1966). Following transfer to the genital tract of the rabbit, two-celled sheep eggs can survive for at least 5 days and develop up to the early blastocyst stage (Averill *et al*, 1955). The survival rate of such eggs in the rabbit is better than those stored *in vitro* 3 days at 7.0°C (Averill and Rowson, 1959). Such an "incubator" offers ample opportunity for successful long distance transportation of sheep eggs (Adams *et al*, 1961; Hunter *et al*, 1962).

## TECHNIQUES OF EGG TRANSPLANTATION

### 1. Selection of Eggs

The eggs should be examined microscopically before transfer to an appropriate recipient. The reproductive cycle of the recipient should correspond to the stage of development of the eggs.

The storage of rabbit eggs at 0°C causes swelling of cells and darkening or roughness of cell membrane, but results in no great deterioration of the zona pellucida. At 10°C prolonged storage may cause coarse granulation, loss of spheroid shape, lack of distinct blastomeres, and marked indentation in the cell mass of the egg. These changes do not necessarily indicate death.

Unfertilized eggs could be stored for limited periods without loss of fertilizability. Shrinkage of the cytoplasm within the zona pellucida due to exposure to glycerol did not appear to affect fertilizability (Lin *et al*, 1957).

The developmental stage of the eggs seems to have an effect on their ability to withstand storage. With rabbit eggs collected 25, 72, and 96 hr. p.c., the younger embryos survived storage best, namely two to four-celled eggs compared to 8 to 32-celled embryos.

### 2. Selection and Preparation of Recipients

Healthy recipients with known fertility should be used. The stage of development of the transferred eggs should be synchronous with the reproductive stage of the recipients. Oviductal eggs should be transferred to the oviduct. Eggs recovered from the uteri of donors should be transferred to the uteri of recipients. A degree of variation from exact synchronization of  $\pm 2$  days can be tolerated in cattle (Rowson *et al*, 1969). Several methods are used to control the time of estrus in donors and recipients; e.g., oral administration 180 mg. MAP for 18 days in cattle (Zimbelman, 1963); daily feeding 60 mg. of MAP in sheep (Dziuk *et al*, 1964); or daily oral administration of 125 mg. of methallibure in swine (Groves, 1967). Two days before the transfer of 2-day-old eggs, the recipient rabbits are injected intravenously with 15 I U of HCG. The injection of HCG mimics copulation in the rabbit by inducing ovulation.

### 3. Technique of Egg Transplantation

Surgical and non-surgical methods have been used to transfer the eggs to the uterus.

**Surgical methods:** The site of laparotomy for egg transfer varies with the species: flank or midventral for cattle (Avery *et al*, 1962b), midventral for sheep and swine (Hancock and Howell, 1961a, b; Dziuk *et al*, 1964; Woody and Ulberg, 1964; Smidt *et al*, 1965; Averill and Rowson, 1958); flank in the rabbit (Hafez, 1970). The eggs to be transferred are drawn up into a fire polished pipette with a minimal volume of medium. The pipette is directed into the oviduct through the fimbriae, or a very small



Fig. 107. Sepsis (ulcer) on the face.



puncture wound in the uterine horn (Fig. 168). When the pipette is in the lumen the eggs and fluid are expelled. The surgical methods will be refined in the future to increase their usefulness in research and possibly genetic improvement.

*Non-surgical methods:* Rabbit eggs were transferred through the peritoneum and without surgery but the rate of success was low. Several attempts were made to transfer bovine eggs without surgery, after insufflation of the uterus with CO<sub>2</sub>. Only a few successful cases were reported (Sugie, 1965; Rowson and Moore, 1966b; Rowson *et al*, 1969).

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Crossbred cow "MAMATA II" (Gir  $\times$  HF, 50%) owned by the Bombay Gow Rakshak Mandali gave a record performance of 53.1 litres of milk at the peak of her third lactation and won the All India Award—"Gopal Ratna". Cross breeding of the indigenous dairy Cattle in India with exotic breeds like Holstein Friesian and Jersey shows a good promise in enhancing milk production. *Rapid growth rate, early maturity, high fertility, regular calvings,* consistency in milk production and longevity are the characteristic features.

## FERTILITY IN FEMALES

A study of available literature reveals that there is a lack of consensus regarding the exact meaning of the word fertility and a corresponding unanimity for a measure of its estimation

Fertility may be defined as the ability of an animal to reach sexual maturity in good time and thereafter be capable of breeding regularly and producing viable young ones commonly expected of that species under the given circumstances

The factors which have to be taken into account for evaluating the herd fertility are enumerated briefly as follows

### 1. Puberty and maturity

The age at which the female initiates her reproductive activities the interval between the first observed oestrus and the first conception and age at first calving which is a valid indication of capability of normal reproduction are some of the important measures for evaluation of fertility The reasons for a delay in the first oestrus and first conception whether due to anovular heats or heats missed heats being too short or weak or due to repeat breeding are additional considerations

### 2 Oestrous cycle and oestral behaviour

Manifestation of oestrus regularity of cycles and its various phases have a considerable bearing on fertility For optimum fertility heats have to be detected in good time and service or artificial insemination has to be synchronised in relation to the time of ovulation Repeat breeding and absence of oestrus without conception having occurred which may be either due to the zygote loss (early

embryonic death) or very short or weak or silent heats, also deserve consideration in evaluating fertility

### 3 Conception rate

The conception rate measures fertility of both the male and female together However this measure of fertility tends to be biased, though without doubt it is least liable to be modified by conscious or unconscious attempts on the part of the breeders to modify For estimation of conception rate only those animals that could attain puberty and sexual maturity would be considered Even closed herds are in a constant state of flux, since adult females are continuously being culled as replacements from the farmbred stock becomes available In the usual mode of reporting no account is taken of the cows that had repeated often and culled due to that reason in estimating conception rates Again such of the growing stock as was culled before the attainment of puberty and maturity is also excluded from consideration for estimating the herd fertility Under these conditions equal opportunity is not available to each individual since animals with a good pedigree or individual performance may be given more leeway while the mediocre ones may be culled at the first opportunity available

### 4 Calving intervals

Calving interval is supposed to be a good indication of the reproductive efficiency but it also has a similar tendency to be biased Initially this estimate would include only such females that have completed at least two normal parturitions Consequently all the females that were culled for some reason or the other before completing two parturitions are automatically excluded

from this consideration. Calving intervals have a tendency to be longer in early age and to decline in adulthood. They may also be influenced by the season of parturition especially in species like sheep, buffaloes and horses which have a more restricted breeding season. Deliberate attempts at extending the calving interval of high producing cows and buffaloes to prolong the lactation period or to obtain their peak yields during the periods of scarcity of milk may not be uncommon. A similar situation exists in case of race horses where all the foals born are arbitrarily stated to complete one year of life on 1st of January every year. Attempts are therefore made to synchronise foalings in relation to this practice. Such attempts tend to cloud the issue since it goes very difficult to find out which of the animals recorded longer intervals as a result of above considerations and which did so as a result of breeding inefficiency or subfertility.

### 5. Breeding efficiency

Several attempts have been made to evolve a single measure of fertility especially in cattle. Wilcox *et al* (1957) proposed comparison of the actual sum total of calving intervals observed with the optimum of 365 days. Dohy (1960) proposed to tag together both the age at first calving and the sum total of calving intervals. Tomar (1965) proposed a similar index in which the actual age at first calving and the calving intervals observed are compared with the optimum age at first calving of 1020 days and the optimum calving interval of 365 days. The first index suffers because it does not discriminate between the heifers having late maturity and those that mature early. Even the latter indices are liable to be biased due to the number of

records under consideration (Tatke and Sane, 1970a).

### 6. Embryonic mortality, abortions, stillbirths, mummified foetuses, prolonged gestations, dystokia etc.

From a review of work of Laing (1949), Stewart (1950) and Hammond (1953) focused attention on the loss of zygotes from the time of fertilization to the actual birth of viable calves. Laing (1949) did not feel that evidence available was a proof for hereditary predisposition. Howel (1959) reported that early embryonic mortality is a major source of apparent female infertility.

In the normal mode of reporting on herd fertility, the above conditions are not usually given due consideration (Bearden *et al*, 1956). Abortions and stillbirths may be due to infective or nutritional factors. However, at least a portion of these and also mummified foetuses occurring in some of the herds have been shown to be due to genetic factors. Cases of dystokia occurring in different species of animals are normally attributed to maternal, foetal or a combination of both and also due to managemental, nutritional and disease factors. However, there is reason to believe that at least a fraction of dystokia cases are directly or indirectly of genetic origin. Prolonged gestation whether accompanying dystokia or not is usually of a hereditary nature.

In many of the estimates of fertility, only normal viable births are accounted for, and no special consideration is given to the above conditions. For example, where mention is made of litter size, number of young ones born per 100 females mated, or calving intervals, it is commonly understood that reference has been made to normal viable young ones

and the interval is between successive normal parturitions. Thus special attempts are required to be made to study hereditary influences.

The various complications cited above hamper a proper estimation of the role of hereditary factors in the expression of fertility. In general environmental factors are supposed to be more important than the genetic ones. This might be due to biases introduced in estimating fertility as discussed earlier. Several of the investigations reported subsequently do reveal that genetic factors may be quite important but again the extent of reliance placed on such findings is questionable. Further if the studies are based only on a limited data or limited aspects of fertility, the value of such findings become even more questionable.

All the above discussion is merely intended to point out the difficulties in discussing the genetic factors involved in control of fertility and reduced fertility.

### INHERITANCE OF PUBERTY AND MATURITY

Puberty is not a simple function of age, size or weight acting independently but rather a stage when an interaction between these three satisfies a pituitary threshold beyond which sexual activity is initiated (Yeates 1965). This clearly means that puberty is a function of postnatal and prepubertal growth and neither age of the animal nor its size or weight alone are an indication of onset of puberty. The age of puberty and its consequences after successful breeding and the age at first calving are therefore under genetic control as also the growth rate of the offspring. Hansel (1959b) felt that age at first oestrus in cattle is markedly influenced by breed and level of nutrition. Van Rensberg

(1957) stated that individuality within breeds is also important and further added that dairy heifers reach puberty earlier than those of beef breeds. Schmaltz (1921) and Hammond (1927) pointed out breed differences in age at first oestrus in cattle. Joubert (1954) provided additional proof from his studies on Jersey, Friesian, Africander and Short horn cattle. The Shorthorns and Jerseys matured earlier than Friesians and Africanders which matured very late.

The age at first oestrus observed at Florida Agricultural Experiment Station (1954) in four different breeds was: Red Poll 381 days, Herefords — 388 days, Brangus — 439 days and Brahman — 455 days. McDowell *et al* (1959) also showed from results of a crossbreeding experiment of cattle that increasing Red Sindhi inheritance appeared to delay puberty. This could well be attributed to natural selection and to adverse environmental conditions obtained in tropics. Eckles (1935) showed that restricted nutrition of heifers is responsible for a delay in onset of puberty. Similar findings have been reported in Holsteins (Eckles 1956, Reid 1953, Sorensen *et al*, 1959) and Swedish Red and White heifers (Hanson 1956). Crichton *et al* (1959) studied nutritional effects on age at puberty in dairy heifers and concluded that though the various treatments resulted in puberty at different ages, the stage of physical development was similar in all age groups. Dale *et al* (1958) reported that Brahman heifers raised at 80°F with 50% to 70% relative humidity reached puberty later than those raised at 50°F.

Trandon (1951) on the basis of his studies on Indian and crossbred dairy cattle reported that early maturity is dominant over late maturity. Jochle and Paleske (1963) reported delayed puberty in Grey

Hounds which from subsequent dam X son matings was found to have a simple recessive mode of inheritance. The bitches which matured late had a better racing performance.

Menge *et al* (1960) reported significant differences in age at puberty of Holstein heifers in different sire lines. Ponkshe (1969) reported differences in 13 Gir cow families in respect of age at first heat (733 to 1118 days), age at first conception (892.25 to 1235.75 days) and age at first calving (1230.78 to 1599.00 days). However these differences were not found significant.

Compere (1964) found that Sahiwal X Ankole heifers in Africa reached sexual maturity at 29.5 months as compared to 35.4 months for their Ankole half-sisters. Wiltbank *et al* (1966) reported from their studies on Hereford, Angus and Shorthorn purebreds and their various crosses that heterotic effects were important in all crosses in respect of age at puberty but were maximum in Hereford X Shorthorn crosses. Onishi (1954) reported that sexual maturity (age at first egg) had a fairly low heritability (0.9 in 1949, 0.023 in 1951) in a flock of Single combed White Leghorn birds consisting of several inbred strains and some crosses between the strains. Inbreeding increased the age but not the variability, while crossbreeding decreased both. A tendency towards patrilinous inheritance of sexual maturity between early and late maturing birds suggested that sex-linked genes might be involved.

Varley and Touchberry (1961) reported consistent but non-significant improvement in age at first calving in 86 first generation crossbred dairy heifers. This was thought to indicate that the trait is largely under the influence of additive

gene actions. From studies on Holstein Freisian X Sahiwal crosses in India, Naidu and Desai (1965) found that in North India, the optimum level of Holstein Friesian blood level to induce early maturity was 3/8 to 5/8 compared to 3/8 to 1/2 in South India. Crosses beyond these levels suffer in growth and maturity which shows that inherited factors as well as adaptability to local conditions influence the age at first calving.

Burkule (1969) reported significant differences in the age at first calving of daughters of three Gir bulls. Tatke and Sane (1970a) reported significant differences in age at first heat and age at first calving of daughters of 14 Khillar bulls. Both the genetic and the phenotypic correlations between the age at first heat and at first calving were positive, high and significant.

Weights at different stages in the pre-pubertal growth period are usually accountable to genetic influence (Naidu and Desai (1965) in crossbred cattle and Tomar and Desai (1965) in buffaloes). Tomar and Desai (1965) reported in buffaloes that weight at birth, 6 months, 1 year and 2 years were negatively genetically correlated with age at first calving and further that the weights at 6 months, 1 year and 2 years showed positive genetic correlation with weight at first calving. This obviously means that selection for early maturity can be attempted on the basis of heavier weights at 6 months, 1 year and 2 years and sires also could be selected on the basis of these traits in their collateral relatives and progeny. Singh (1966) stressed that lower age at first calving at optimum body weight needs to be considered since these animals can also be predicted to have higher lifetime breeding efficiency.

Table 71

## HERITABILITY ESTIMATES OF DIFFERENT REPRODUCTIVE TRAITS

**1 Age at First Heat**

Heritability $\pm$ S E	Breed	Species	Workers
0.075	Haryana	Cattle	Choudhury <i>et al</i> (1965)
0.91 $\pm$ 0.665	Khillar	Cattle	Fatke and Sane (1970a)

**2 Age at First Egg**

0.17 $\pm$ 0.24	White Leghorn	Poultry	Husain and Singh (1964)
0.15	White Leghorn	Poultry	Kumar and Kapri (1967)
0.09 and 0.023	White Leghorn	Poultry	Onishi (1954)

**3 Weight at First Egg**

0.52 $\pm$ 0.32	White Leghorn	Poultry	Husain and Singh (1964)
0.72	White Leghorn	Poultry	Kumar and Kapri (1967)

**4 Age at First Fertile Heat**

0.386	Haryana	Cattle	Choudhury <i>et al</i> (1965)
0.536 $\pm$ 0.352	Ankole	Cattle	Compere (1965)
0.27 $\pm$ 0.12	Murrah	Buffalo	Singh and Dutt (1964)

**5 Age at First Calving**

0.16 $\pm$ 0.29	(Bangalore) Red Sindhi	Cattle	Amble and Jain (1966)
0.30 $\pm$ 0.16	Red Sindhi	Cattle	Stonaker (1953)
-0.09 $\pm$ 0.17 (Hosur)	Red Sindhi	Cattle	Amble and Jain (1966)
0.70	Tharparkar	Cattle	Sunderesan (1963)
0.66 $\pm$ 0.24	Kankrej	Cattle	Amble and Jain (1966)
0.5043 $\pm$ 0.0198	Sahiwal	Cattle	Batra and Desai (1964)
-1.24 $\pm$ 0.58	Gir	Cattle	Amble and Jain (1966)
0.1976 $\pm$ 0.088	Gir	Cattle	Mangurkar (1967)
0.564 $\pm$ 0.376	Gir	Cattle	Shukla (1965)
0.8511 $\pm$ 0.017	Gir	Cattle	Burkule (1969)

Puberty in sows is controlled by genetic, nutritional and seasonal influences as well as interactions between factors. Phillips and Zeller (1943) found that the mean age at puberty for the small and large type Poland China gilts was 208 and 199 days respectively. Burger (1952) found no difference between Large Black, 219.2 days and Large White, 216.8 days and thought that genetic differences between families contribute more variability than breeds since litter mates often show first oestrus either on the same day or within a period of few days.

Kascenko and Porcenajev (1964) reported in Large White breed of pigs, that inbreds had slower rate of growth and consequently matured late.

In short, the available work reviewed here makes it quite clear that early puberty and maturity in the different species of farm animals have a close bearing on the weight at these two stages. The positive and significant high correlation between age at first heat and first calving indicates that selection applied for the earlier expressed trait will also be helpful in the genetic improvement of the trait expressed later. It has also been shown that selection can be based on the earlier weights i.e. at 6 months, 1 year and 2 years as these have a positive genetic correlation with weight at first calving and a negative genetic correlation with age at first calving. However this would be possible only if accurate pedigree records and records of weights are maintained. From the work reported so far, it can be inferred that in animals with good fertility, the interval between puberty and sexual maturity is short.

### **INHERITED ANATOMICAL AND PHYSIOLOGICAL DEFECTS LEADING TO DISTURBANCES IN FERTILITY DURING PRE AND POST-PUBERTAL LIFE**

A trained clinician can help a great deal in exposing cases of developmental abnormalities which are likely to reduce fertility. This is especially true in large animals due to the ease with which the entire genital tract can thoroughly be palpated during rectal examination. A knowledge of such of the defects as are known to have a hereditary basis and availability of reliable and complete pedigree records will go a long way in timely detection and elimination of suspect carriers and prevent their spread.

The inherited abnormalities involving the genital tract include the following (1) Gonadal hypoplasia, (2) White heifers disease, (3) Freemartins, (4) Hermaphroditism and (5) Cystic ovarian degeneration. These probably have a genetic origin and are occasionally encountered.

### **GENETICAL CONTROL OF OESTRUS, OESTROUS CYCLE AND OESTRUAL BEHAVIOUR**

The variations in oestrous cycle length and the hereditary influences conditioning them may not be of great economic importance unless of course they are gross. However they may be of practical importance especially in Artificial Insemination work. Expression of oestrus i.e. intensity of perceptible physical, physiological and psychical symptoms of heat are however of great practical and economic importance since complete failure to detect heat or its delayed detection means delayed breeding by one oestrous cycle and a corresponding increase in the length of the calving interval. The interval between the first



postpartum heat and conception often includes long intervals which can best be described as double cycles (35 to 45 days), triple cycle (35 to 65 days) and so on. A portion of this type of cases may no doubt be due to zygote or embryonic losses due to infectious factors but a sizable portion can only be due to short, weak, silent, and unovular or missed heats and due to immunological factors. When investigating genetic control of oestrus and allied phenomena, these aspects need special investigations.

### Breeding Season

Sexual activity is restricted to particular seasons in wild animals and domestication tends to lengthen the season so that some species and even breeds of the same species may be monoestrus and with domestication they tend to be seasonally polyoestrus and ultimately polyoestrus. There is reason to believe that this might be due to hereditary control. The control mechanism appears to be acting through the eyes on the pituitary and as some recent work appears to show via the pineal gland. Species have been classified as either long day breeders i.e. the reproductive activity is stimulated by increasing day light hours while the others designated as short day breeders tend to initiate the reproductive activity as the days shorten. Even in a given species like sheep, breeds differ in the length of breeding season. Breeds of sheep like Dorset horn tend to have a short breeding season extending over one or two cycles only, whereas others like Merino have much longer breeding season and proportionately a very short anoestrus season. Selection carried out in Santa Gertrudis breed shows that it is possible to breed for a restricted season. Environmental

factors like presence of ram with the flock or its first introduction after a withdrawal at the end of the last breeding season and availability of fresh growing grass after the first summer showers hastens the onset of breeding season and may delay it under adverse conditions. The residual differences between the different breeds can be largely due to genetic factors.

De Baca *et al* (1954) reported that during 1951 and 1952 the average date of first heat in the breeding season in different breeds was Hampshires — 6th August, Romneys — 21st August and Southdowns — 6th September. No breed differences were observed in the first crosses of Hampshire, Border Leicester, Romney and Cheviot rams used on Lincoln x Rambouillet ewes but when the  $F_1$  ewes were backcrossed to rams of their sire's breeds, the onset of oestrus was influenced by breed. These results indicate genetic differences in onset of breeding season.

Scott *et al* (1959) studied reciprocal crosses between Basenji and Cocker Spaniels. The former exhibit only one cycle a year and the latter breed at any time in the year but approximately at six months intervals. The  $F_1$  progeny showed a high degree of variability in occurrence of oestrus. Initially two types of intervals were observed, a long one of 9 to 11 months and a short one of 2 to 3 months. Interval of other lengths were also observed though none of the crosses exhibited two long intervals consecutively as in Basenji dogs. The  $F_1$  studs were then backcrossed to purebred dams and also interbred with crossbred sisters. In the back cross to Basenji, 9 out of 19 bitches showed Basenji type of cycle as did 3 out of 32 in the  $F_2$  generation obtained by inter-

breeding This would seem to indicate that the Basenji type of cycle is determined by a single recessive gene but a multifactorial hypothesis cannot be over ruled

Jochle and Paleske (1963) reported delayed puberty and long (upto 9 to 12 months) intervals between oestrous periods in a group of Greyhound bitches About half the bitches had a much better racing performance than those maturing at the normal age and came on heat every six months It was found from Dam  $\times$  Sire matings that both the males and females are carriers for this defect inherited as a simple recessive

### Inter oestral length

Chapman and Casida (1937) studied the causes of variation in the length of oestrous cycles When all cycles were considered the individual tendencies were masked However when only cycles between 17 to 24 days were taken into account each animal had a definite individuality in length and 41% of the variance was between individuals Roberts (1959) concluded that individuality of cows and heifers was the principal cause of variation in the length of oestrous cycles Olds and Seath (1950 and 1954) found that in The Kentucky Agricultural Experiment Station herd between cows variation was significantly more than within cow variation and this tendency was enhanced when only cycles between 17 to 27 days were considered Hammond (1927) described marked individual differences in the length of oestrus in cows Anderson (1941) recorded breed differences from his studies on East African Zebu and grade cows in Kenya Asdell (1952) reported in Beltsville herd estimate of re

peatability of 0.18 Bhasin (1969) reported in Haryana cows a repeatability estimate of  $0.2034 \pm 0.1643$  Ponkshe (1969) reported highly significant differences in the length of oestrous cycle of 13 Gir cow families

Anderson (1944) described breed differences in the length of oestrous cycles Brackel *et al* (1952) reported that Brown Swiss cows at Ohio have shorter cycles than other breeds

The length of oestrous cycle is also under considerable influence of non genetic factors such as age (young animals tend to have shorter cycles than adults) and season However the individuality of animals has to be kept in mind

### Phases of oestrous cycle

Not much information is available regarding the genetic control of the duration of oestrus and other phases of oestrous cycles in farm stock This may have considerable importance since short duration of oestrus specially when heats commence at odd hours may be a contributing factor in prolonging the interval between the first postpartum or pubertal heat and conception and hence deserves greater attention

Willemsse and Boender (1966) reported in Large White gilts that oestrus in swine can be divided into three periods (a) a period during which only the boar can evoke the standing reflex (b) a period during which the standing reflex can be evoked by the inseminator by pressure on the back and sides of the sow and (c) a second period similar to the first Though individuals differed in the actual duration of oestrus they tended to show a constant ratio of 1.4:1 between

these three periods. Variations between groups of sisters were greater than those within groups of sisters and variations within individuals were smaller than those between individuals 147 of 157 gilts examined confirmed to this pattern, the remaining 10 gilts did not stand for the inseminator. Two of these had cystic ovaries and 8 attained puberty after 270 days of age. Three of these gilts were two full sisters and a half-sister, sired by one boar.

With such evidence in favour of genetic control of the various phases of oestrous cycle, it is particularly essential to study the genetics of short oestrus periods since they pose a problem even when detected as regards the synchronisation of service in relation to the time of ovulation which is commonly related to the end of oestrous in cattle and buffaloes.

### Intensity of heat

Rottenstein (1958) studied 2343 oestrous periods of 724 primiparous heifers sired by 38 bulls of three breeds in a progeny testing station in Denmark. The strength of oestrous was expressed on a scale ranging from 1 (doubtful) to 4 (stronger than normal). The average values for each of the 38 daughter groups varied from 1.82 to 3.00 and differences between different daughter groups were significant. Jersey heifers had an average value of 2.69 compared to 2.48 and 2.50 for the Danish Friesians and Red Danish heifers, and this difference was significant. Post-oestrous bleeding was observed in 27% oestrous periods and the differences in incidence between different daughter groups were significant.

Lagerlof (1951) found that weak expression of heats is a problem in certain

breeds of cattle. Simmenthalar cattle in Switzerland, Telemark cattle in Finland and Swedish Highland cattle as a rule have intense and pronounced heats detectable even under adverse conditions. In Swedish Red and White cows on the other hand, the expression of heat is so weak that detection of heats is very difficult especially during winter when all the cows are stalled. It was also felt that the greatest problem facing the Swedish A.I. Association was that of weak heats. In an analysis of data of 2,30,000 cows, the percentage of diagnosed pregnancies in cows exhibiting pronounced heat was 57%, indistinct cases 45% and very indistinct heats 26%. Lagerlof (1951) stated that occurrence of weak heats was more common in Swedish Red and White breed than in Swedish polled cattle and in the former the incidence was higher in winter. Brackel *et al* (1952) recorded less intense heats in Brown Swiss cows at Ohio as compared to the other breeds. Donald and Anderson (1953) stated that red, roan and white cows have less intense heats than black cows. Grimm (1954) concluded that follicular atresia might be inherited. Rottenstein and Touchberry (1957) analysed the data of Danish progeny stations and found that repeatability of expression of heat was 0.29 and heritability 0.21. Heifers conceiving at first insemination had more distinct symptoms of heat than those requiring two inseminations and those requiring three inseminations had even weaker heats. Such studies give a clear indication that intensity of heats is at least partially heritable.

Swenson (1963) studied 947 daughters of nine sires and found that 63 daughters of one bull had significantly lower fertility which was ascribed to their exhibiting slight or no signs of heat. Labrecq

wai *et al* (1963) studied 3076 ovulations of 728 Holstein cows and reported an incidence of 23.7% quiet ovulations. Both sire lines and season had significant influence on frequency of quiet ovulations. A negative and significant correlation was found between their occurrence in heifers at 12 months of age and interval since calving. Kumar (1968) from his studies on Hariana cows reported significant differences between cows in length of the insemination period (postpartum interval to conception minus postpartum interval to first heat). This interval may be increased either as a result of short heats that missed detection or weak or silent heats in addition to the other well known factors like early embryonic deaths due to genetic or infectious causes. The number of heats missed (difference between heats detected during the insemination period and double or treble cycles etc.) was maximum in heifers (3.29) and was lowest after the fifth calving (0.74). These differences were significant between cows when the pubertal heats were taken into account but not between subsequent calvings. The heritability for this trait was  $0.22 \pm 0.23$ . This may mean that the tendency for short heats or weak and silent heats is at least partly inherent. Odegard (1965) reported that heritability for intensity of oestrus was 0.05 in cows.

### Abnormalities of ova

The bulk of the investigations on the gametic abnormalities are confined to sperm since they are available for examination in large number and with ease. The collection of ova at the proper time is more complicated and in uniparous animals only one ovum may be available per animal every three weeks

or so and even in a prolific species like swine not more than a couple of dozens.

Amongst attempts made to identify and classify abnormalities of ova following have been mentioned: (a) irregularly shaped ova (b) ghost ova (with empty zona pellucida) with or without rupture of zona pellucida (c) ova with ruptured vitellus, shrunken vitellus or cytoplasmic fragmentation and (d) ova showing irregularities or incomplete cleavage in different species of animals viz cows (Winters *et al*, 1942; Kidder *et al*, 1954; Pitkanen and Ivankov, 1956), sheep (Dutt, 1954; Dutt *et al*, 1959; Hulet *et al*, 1956; Averill, 1955, 1958) and pigs (Squires *et al*, 1952). Hart (1956) considered that the ewe rather than the ram may be the cause of infertility in sheep from his studies on 866 reproductive organs of Corriedale type of ewes over a six year period. More than a thousand ova were collected. The normal ova were classified in three basic types each. Similarly the abnormal ova were classified as (a) ova showing involution (b) ova showing vacuolation and (c) unclassifiable ova. These abnormalities of ova are responsible for reproductive losses for a period of 9 to 11 days ovulation i.e. until primary implantation. During the 1955 breeding season of the 220 ova recovered from 252 ewes 17.3% were abnormal. Involution was responsible for 63.2% of abnormal ova produced and was seen to affect only single celled eggs thus prevented fertilization. Vacuolation can prevent fertilization or destroy the fertilized egg before implantation.

The morphological abnormalities of ova are difficult to interpret. Averill (1958) reported that none of the six ova classified abnormal could develop after transfer. In contrast Hancock and Hovell (1961) found that nine abnormal sheep ova developed after transfer.

Pitkjanen (1958) mentioned that sheep ova look much less uniform than pig ova and swine workers may misinterpret if they examine sheep ova. Ova which appeared normal when examined fresh may show abnormality just as apparently abnormal ova may show normal features after fixation and staining (Hancock and Hovell, 1961).

Hafez (1961) stated that the ova of placental mammals varied between 60 to 80  $\mu$  in diameter (vitellus alone). The size of the ova varies within breeds, within individuals and also within the same animal. He postulated that the morphological characters of the gametes are directly or indirectly determined by the genotype of the animals. The abnormalities of ova observed were of size and shape, degree of cytoplasmic granulation and early fragmentation etc. The abnormal shapes were described as helmet kidney, amoeba, oval and paramecium or hammer head types. Such types may be responsible for failure of subsequent fertilization or embryonic development.

of services required per conception) have received maximum attention as a simple measure to express fertility. The former is usually used when bulls are to be compared or other genetic and non-genetic factors influencing fertility are to be studied without reference to individual females. The latter is usually used when individual females or the same female from lactation to lactation is required to be compared. However the difference in these two measures is one of form alone and they can be interchangeable. A review of literature tends to show that this reproductive trait is more under the influence of non-genetic factors than the genetic ones. However a few of the carefully investigated studies do show that genetic factors are also involved and may play an important role. Edwards (1947) and Stewart (1950) recorded breed differences in conception rates. Shorthorn cows show consistently high conception rates. Muller (1957) reported from Austria from a study of over 1,28,000 cows of six breeds, that significant breed differences were found in

next year Trimberger and Davies (1945) from their studies on 133 virgin heifers and 199 cows stated that repeatability for this trait was near about zero. Korkman (1947) attributed only 5% of the differences during different conceptions to permanent differences between cows. Olds and Seath (1950) reported from an extensive study on more than 6500 cows a low ( $0.084 \pm 0.012$ ) but significant correlation between the conception rates for the consecutive years of the same cows and the predictability was almost of the same order when herds were considered as units.

Casida (1950) investigated two herds bred exclusively by artificial insemination and reported that only about 14% and 18% of the total variation in conception rates was permanent. Culling of repeat breeders in one of the AI Centres would have reduced the incidence of repeat breeding in the next year by only about 8%. Asdell (1952) also reported very low repeatability estimate for non return to first heat (0.027) and services per conception. Rendel and Venge (1961) studied genetic and environmental factors influencing fertility of 264 Swedish Red and White bulls at the AI Centres in Sweden. There was a correlation of only 0.075 in respect of conception rate of paternal half sibs. Singh (1964) reported from a study of 867 calvings of 207 Tharparkar cows the repeatability estimate of 0.099 and considered it too low to be of any practical importance. Deshmukh and Taylor (1969) reported in two herds of Deoni cattle in Maharashtra repeatability estimates of  $0.2009 \pm 0.0857$  and  $0.0408 \pm 0.0817$  respectively.

Manjala (1961) conducted a very exhaustive study of about 200 000 service

periods from Finnish recorded statistics 120 000 from Finnish AI statistics and about 180 000 from Swedish AI statistics. The heritability estimates of various measures of fertility were generally low with median value of 1 to 2%. The heritability values of heifers did not differ much from those of cows. He advised that the number of services per conception in conjunction with frequency of calving intervals longer than 15 months appear to be the best measure of fertility. Frequency of culling due to sterility gave heritability estimates of similar order to other estimates. Non return rate and final conception rate were almost of the same merit in determining the hereditary portion of fertility (1.5% each). The former is obtained earlier and being easy to obtain may be preferred.

Shannon (1965) reported that sampling variation due to the binomial nature of data accounted for 88.5% of the observed variation in first insemination conception rate, 43.5% to variations between herds and 11.3% to variations between technicians. Only 9.7% of the observed variation could be attributed to cows themselves. He also concluded that the maximum observed heritability in such type of data would be only about 0.099. Spears *et al* (1965) also studied variations in conception rates in dairy cows from 20240 inseminations over a four year period and reported that 79.6% of the total variation was due to sampling error, 1.4% due to year differences, 4.6% due to district differences, 7.5% due to herds within district and 1.9% due to year  $\times$  herd  $\times$  district interaction. Such results indicate that nongenetic factors exert a greater sway over conception rate than the genetic ones.

members of the same cow family was only about 15% which might be the reason for the differences being negligible. Hauserman (1952) studied the records of Württemberg Spotted Mountain cattle and compared the pedigrees of 11 families of poor fertility and 9 of average fertility. 50% of the cows showing poor fertility suffered from disturbed ovarian functions, which would well be attributed to genetic factors. Mack (1953) believed that considerable differences in family averages of fertility were due to hereditary factors. Legates (1959) studied the number of services per conception in different herds in North Carolina and concluded from estimates of repeatability and heritability that genetic factors have little influence on this trait.

Bachner (1961) analysed fertility of five lines of a German Simmental cattle herd between 1932 to 1959. In lines S and C, the number of inseminations per conception were 1.30 and 1.83, respectively. In the early days of this breed, all heifers reaching a certain level of growth were served and those that failed to conceive with a maximum of two services were culled. In the two lines, 3.33% and 22.70% of the heifers were culled and the proportion of cows culled after several calvings were 8.3% and 43.7% respectively. Genetic control of fertility was postulated because only one bull was in use at a time in this herd. Van Rensberg and De Vos (1962) compared the frequency of ovulation failures (which may lead to increased services per conception or a low conception rate) and found no overall difference between Friesian and Africander cattle. The former was introduced exotic stock and the latter native adopted stock. Examination of records of four

breeds revealed that the latter breed had high reproductive and the remaining had low eff

Such records of lack of conclusive differences between families may be due to one or more of the following factors: (a) The individuals within a cow family may belong to widely separated periods over several generations and the fluctuations in the environmental and managemental conditions may be so large that the genetic differences may tend to get masked. (b) The number of cow families at a particular period is usually much larger than the number of sire families in a herd i.e. the average number of progeny per cow family becomes smaller to give any valid estimate of the cow component. (c) No attempt has been made to retain the individual foundation cows amongst the successive generations of that family at the absence of selection and use of a controlled breeding system for this purpose. (d) Failure to get sufficiently different

## **Inheritance of multiple births in monotocous animals**

### **INHERITANCE OF TWINS**

Since sex determination is controlled by X and Y type of chromosome, fertilisation produces in a sufficiently large size about 50% males and 50% females, in case of dizygotic twins, one would be male and the remaining female, unlike sexed. Any increase in the frequency of like sexed twins is not ascribed exclusively to be due to the presence of monozygotic twins.

Lush (1925) scrutinised the records of every set of a twin dropp

Table 72

## HERITABILITY ESTIMATES OF CONCEPTION RATES IN CATTLE

Heritability	Breed	Worker
$-0.15 \pm 0.09$ } $0.08 \pm 0.09$ }	—	Curman (1955)
$-0.01$	—	Rognom and Bette (1960)
$-0.018$ (1st conception) $0.117$ (1st conception) $0.5 \pm 0.26$ (19 days NR)	Tharparkar	Singh (1951)
$0.10$ (Heifers 1st insemination CR)	—	Lee Roy and Hahn (1963)
$0.099$ Heifers 1st insemination CR)	—	Shannon (1965)
$0.13 \pm 0.37$	Haryana	Kumar (1968)
Culling Rate (in cattle)		
$0.095$	—	Inskip and Tyler (1961)
$0.31$ (8 yrs old 1st record cows) $0.21$ (4 yrs old 2nd record cows) $0.63$ (3 yrs old 1st record cows) $0.56$ (1 yrs old 2nd record cows)	Brahman Brahman Brahman Shorthorn	Deese and Koger (1947)
$0.12$ (Pooled estimate)		
Stenility (Barrenness) in sheep		
$-0.01$ Scottish Black face sheep $0.07$ Welsh Mountain sheep		Purser (1965)

Kansas Experiment Station Holstein Friesian herd and traced the genealogy back to the sire Hengerveld De Kol and his grandson Cronus. Daughters of these bulls produced significantly more twins than the herd average of 8.84%. Johansson (1932) cited several workers who had studied the pedigrees of twin families to trace the twinning tendency. In one particular herd having greater frequency of twin births the daughters of bull Prins Lejonhjärta had a higher incidence of twin births and 18 grand daughters also exhibited a similar tendency. It was suggested that this bull transmitted the gene for multiple births to his sons since all of them sired daughters which had higher frequency of twin births. Rab (1937) re-

corded that twinning occurred frequently in 19 families of Yellow Franconian cattle and proposed a recessive mode of inheritance for twinning tendency. Pfau *et al* (1948) studied a Holstein Friesian herd in which 90% of the cows belonged to 21 families of these twin births were not observed in 10 families. The rate of twinning in the remaining families ranged from 2.63 to 18.18%. Ten of the 19 sires used in the herd sired daughters producing twins (range 2.27 to 14.29%). It was postulated that a few autosomal genes controlled the predisposition to twinning. Hadre (1942) concluded that twinning in cattle was inherited as a sex linked dominant. Weber (1944) however felt that tendency for twin ovulations was determined by a



single recessive gene and all mothers of dizygotic twins are homozygous for it. Johanson and Rendel (1966) have com-

piled data for a number of breeds of cattle and estimated incidence of monozygotic twins as follows:

Breeds	Total No. of births	% twin births	MZ pairs as % of like sexed pairs
Simmental	12,625	4.61	$6.00 \pm 7.43$
Holstein-Friesian (USA)	18,736	3.08	$8.60 \pm 7.62$
Swedish Friesian (SLB)	24,670	3.32	$6.81 \pm 3.19$
Swedish Red and White (SRB)	53,554	1.85	$11.05 \pm 3.31$
Swedish Polled (SKB)	3,751	1.81	$26.78 \pm 10.06$
Jersey (New Zealand)	87,926	1.02	$16.60 \pm 6.06$

The incidence of twinning and multiple births in Indian cattle is much low. (Bhattacharya *et al.*, 1956). Incidence is rare in buffaloes at Aarey Colony, one pair in over 50,000 calvings (Marathe, 1970).

The differences in frequency of twins between different breeds were significant and were probably genetically determined. The overall estimate for monozygotic likesexed twins was  $10.61 \pm 1.84$  and none of the breeds deviated significantly from this average.

The chances of twin births in case of cows with recorded twin pregnancy are three to four times higher. In the Swedish Red and White cows, daughters of dams born as twins had a frequency of 3.72 twin parturitions compared to only 1.77 for others. Korkman (1948) analysed the same data and found no correlation between dams and daughters but about 0.1% between full sisters indicating nonadditive type of genetic control.

#### Inheritance of multiple births in polytocous species

Fertility in polytocous animals presents a problem different from that in

the monotocous species. In the latter, formation of a viable egg, subsequent to conception and ultimate birth of a viable young one is a valid indication of the female fertility. In the former however, production of optimum number of viable young ones commensurate with the age, breed and plane of nutrition. In swine, the litter may vary in number from 2 to 25. Single litters are usually rare and litter size is generally considered as a nongenetical trait, depending largely on chance since majority of reports have ascribed low heritability values to it. Cockerman (1952) analysed data on 1080 litters from 12 inbred lines of Poland China and one of Danish Landrace pigs and reported that heritability for number of piglings farrowed was  $-0.11 \pm 0.07$ . Sampling error or a rather delicate balance of over dominance and selection was thought to be the explanation.

Newman (1963) concluded from his studies on Lacombe breed of pigs that ovulation rate was subject to a high degree of genetic control but was not highly correlated with litter size at day 50 and day 100 of pregnancy.

Table 73  
TWIN AND MULTIPLE BIRTHS IN SHEEP

Breed	No o births	No of lambs per lambing	% of multiple births			
			Twins	Triplets	Quadruplets	Quintuplets
Oxford Down	6 838	1 52	45 8	3 4	0 06	—
Shropshire	16 765	1 48	43 0	2 4	0 10	0 01
Cheviot	25 779	1 46	13 6	1 3	0 06	—
Landrace (I)	7 277	1 66	54 8	5 2	0 33	—
Landrace (G)	1 722	1 43	42 2	0 5	—	—

Johansson and Hansson (1943) from their extensive studies on twin and multiple births reported considerable difference in Swedish breeds of sheep (Table 73)

Rendel (1956) studied 30989 births of Cheviots Oxford Down Shropshire and Swedish Landrace sheep between 1925-42 from 425 sires out of 5093 dams. Against the overall frequency of twin births of 51.1% the Landrace recorded highest frequency of 69.1% the pooled estimate of heritability of multiple births was 0.113

Desai and Winters (1951 a) reported in sheep that twin born ewes constantly keep themselves at a higher level in lambing average than single born ewes and such differences widen markedly between third to sixth year of age. These differences between the two groups were highly significant. Desai and Winters (1951 b) reported from their studies on nine groups of sheep at four farms that heritability estimates of litter size by intrasire daughter dam regression ranged from  $0.2764 \pm 0.0045$  to  $0.2825 \pm 0.0637$  when only corresponding records of daughter and dams were considered they ranged between  $0.2764 \pm 0.0045$  to  $0.3379 \pm 0.0608$ . The pooled estimates for all groups were  $0.03606 \pm 0.0102$  and  $0.0736 \pm 0.0152$  respectively.

Donald (1963) reported considerable breed differences in fertility levels of sheep. Finnish Landraces and Russian Romanowsky breeds had outstanding fertility. One of the rams of Finnish Landrace breed was one of the litter of seven and the other from a litter of six. When 10 of the imported females were bred they produced 28 lambs.

Amble *et al* (1962) reported in Beetal goats the heritability of multiple births of  $0.15 \pm 0.10$  by intrasire regression of daughters on dams.

Soller and Kempenisch (1964) from a study of 1338 litter born to 583 polled and 354 litter born to 166 horned Svanen nanny goats during 1952 to 1962 in 50 herds reported that the average litter size of polled nannies was 2.03 compared to 1.91 for the horned ones. Frequency of triplets was 19.7% and 15.0% respectively. The weighted average was 0.093 kids/litter in favour of polled nannies. In 38 herds out of 51 studied the polled nannies recorded significantly higher triplets than the horned ones. Since polled females are almost all heterozygotes it was concluded that the gene for polled condition may increase litter size in heterozygous condition.

Van Oers (1961) studied litter size of 3530 daughters of 22 boars. Heritability

ties for families over 50 daughters for litter one to four were 0.21, 0.10, 0.11 and 0.06 respectively. It was concluded that boars should be progeny tested for improvement of litter size.

Purser (1965) from his studies on inheritance of fertility in sheep in Scottish Blackface and Welsh Mountain sheep, advocated for greatest improvement, selection on the basis of litter size at birth.

Ahmedov (1964) reported that lesser proportion of black Karakul ewes remained infertile than grey ones. The black ewes had higher lambing rates and low mortality.

Evans and Turner (1965) reported in Australian Merino sheep that in four flocks, ewes with haemoglobin type A had fewer lambs born and weaned, than ewes with Type B or AB. There was little difference between the latter two groups. The haemoglobin variants are genetically controlled.

Ragab and Askar (1954) from a study of 1113 Ossimi lambs that twinning increased with age and reached maximum at sixth and seventh lambing. The type of lambing of dam had no effect; neither was there a difference between inbred and outbred ewes. There was however a significant but indirect effect of sire.

Table 74  
HERITABILITY OF MULTIPLE BIRTHS

Heritability $\pm$ S.E.	Breed	Species	Worker
1. —	Ossimi	Sheep	Ragab and Askar (1954)
2. 0.14	Scottish Blackface	Sheep	} Purser (1965)
3. 0.16	Welsh Mountain	Sheep	
4. $0.0306 \pm 0.0102$ $0.0736 \pm 0.0152$	—	Sheep	Desai and Winters (1951 b)
1. $0.15 \pm 0.10$ Littersize at birth	Bcetral	Goats	Amble <i>et al</i> (1962)
1. $0.59 \pm 0.29$ $0.20 \pm 0.15$	Mangala	Swine	Stockhansen and Boylan (1966)
2. $0.15 \pm 0.084$	Hungarian White	Swine	Farencz (1965)
3. $0.08 \pm 0.04$ Total Littersize	—	Swine	Urban <i>et al</i> (1966)
1. $-0.11 \pm 0.07$	Poland China	Swine	Cockerman (1952)
2. $0.56 \pm 0.20$	— (inbred)	Swine	Noland <i>et al</i> (1966)
3. 0.28	Danish Landrace	Swine	Jensen (1965)
No. of viable offspring at birth			
1. $0.11 \pm 0.23$ $0.51 \pm 0.20$	Poland China	Swine	Noland <i>et al</i> (1966)
2. 0.20	Danish Landrace	Swine	Jensen (1965)

### Fertility in Poultry and other birds

Fertility in poultry and other birds is even more difficult to define than in mammals. It must take into account both the total number of eggs produced and whenever the eggs are required for hatching purposes the hatchability of eggs. For the commercial egg producers selling eggs for table purposes only the number of eggs produced is of importance while for breeders and hatchers the hatchability is more important.

Tarvin *et al* (1960) described an experiment to study inheritance of fertility of White Leghorn hens. Two groups of cockrels and four randomly chosen groups of hens were arranged for diallele crosses. The parents of the first group of cockrels had high fertility (sires 72.9% and dams 96.1%) while those of the other groups had low fertility (19.1% and 38.7%) respectively. Analysis of performance of progeny indicated that fertility differences in poultry have

28.4% for inbred dams. 51.7% of the embryonic deaths occurred between 16 and 34 days of gestation.

Studies were carried out on the Jersey and Holstein cows and random experimental matings were arranged between unrelated cows and bulls (Gregory *et al* 1915 and Mead *et al*, 1916). The studies apparently yielded some evidence for autosomal recessive genes for female sterility. It was seen that though fertilization occurred and cleavage commenced there was no normal growth to term (Gregory *et al*, 1951). Sterile Jerseys expressed normal oestrus symptoms but the sterile group of Holsteins usually showed complete absence of symptoms of heat and about three out of five cycles were observed to be abnormal. This could be attributed to genetic variation in levels of hormone production. Mead *et al* (1916) estimated the gene frequency of sterility in two breeds as Jersey = 0.333% and Holstein = 0.083%. The two types of sterility were believed to be due to different

viding suitable intrauterine environment for the developing zygote.

Kidder *et al* (1954) studied this problem in four breeds of Holstein and Jersey cows. It was revealed that bulls with low fertility were responsible for higher embryonic death rates than those of high fertility. Hawk *et al* (1955) found that the bull had a highly significant effect on the rate of embryonic death of cows to which he was mated.

Dawson (1958) investigated causes of stillbirth in cattle. In six outbreaks in dairy herds during the year, one Friesian herd showed symptoms of hypothyroidism, while the remaining (1 Guernsey, 1 Short Horn X Jersey and 3 Jerseys) showed hyperthyroidism. Data from these five herds showing high incidence of retained placenta suggested a progesterone deficiency with hyperthyroidism. The syndrome appeared to have familial relationship. Stevens and King (1968) from their investigations into the high incidence of mid-term abortions in a herd of Holstein-Friesian cows in Ontario analysed the pedigree of 9 cows, 6 of which had at least one abortion; and 2 bulls which were used extensively in this herd and which could be traced back to one common ancestor. The abortions usually occurred in the 5th month of gestation and often was a sequelae to mummification of foetus. The condition was suspected to be due to presence of an autosomal recessive gene.

Pond *et al* (1960) from a study of 162 Berkshire, 138 Chester White and 251 Yorkshire litters involving 5316 live births in swine, reported highly significant differences among breeds in litter size, number of still-births and number of mummified foetuses. Berkshire sows had the smallest litter size and greatest

frequency of mummified foetuses. Of 97 litters that had mummified foetuses only 29 also had still births and only 31 out of 219 litters that had still births also had mummified foetuses. This indicated no causative correlation between these two conditions. Hafez (1960) reported from a study of 36 Palouse Sows mated to five fertile boars and slaughtered at either 38 or 100 days post-service that the total prenatal loss was, 16.22% lost ova, 15.83% embryos and 8.72% foetuses. These sows whose progeny showed hydroadorsal defect were related and could be traced to two particular boars. It was concluded that genetic control may be involved in foetal atrophy and prenatal mortality. Perry (1960) studied embryonic loss in swine as measured from the differences in the number of corpora lutea present and the number of ova not represented by embryos in 36 gilts during second month of gestation. The gilts were arranged as full sisters and half sisters and these differences were studied. It was concluded that the differences in percentage of ova lost were due to the effect of the sire. A similar study was carried out on 16 Large White Sows in a double reversal experiment. Sire effects on percentage of ova lost were brought out from this study.

Coneally *et al* (1963) estimated that less than 8% of the zygotes in cattle die due to genetic load. Laverge and Vissac (1963) reported that incidence of a condition "sheep head" was 5.4% in 84 progenies of one Limousin bull. This abnormality was due to an autosomal dominant gene with incomplete penetrance. Perinatal mortality of abnormal and normal calves of this bull was  $32 \pm 12.2\%$  and  $4 \pm 1.4\%$  respectively. Embryonic mortality from inseminations of this bull was  $8.62 \pm 0.44\%$  compared

Hornday (1947) suggested that foetal mummification in cattle has hereditary bias as seen from his studies on four generations of cow families since it was seen in same cow families from generation to generation. The condition mummified foetus may be inherited as a simple recessive or have complex inheritance, though non-genetic factors may also be responsible (Deaton *et al*, 1969; Roberts, 1962).

Grootenhuis (1956) from a study of statistical data of 10,000 mares and pathological investigations of 105 foetuses and foals which died at birth, concluded that abortion, still birth and disease in foals form a syndrome for which heredity is partially responsible.

Buechi (1957) analysed the 1950-51 records of 146 Swiss Goat Breeders Associations of three polled breeds, i.e. Saanen, Toggenberg and Oberhasli — Brienz and three horned breeds i.e. Grisons-striped, Gems farbige Gebirgezige (chamois coloured) and verzasca. In polled breeds, the secondary sex ratio averaged 64.5% males compared to 54.8% in the horned breeds. The differences between the normally expected ratio were significant. The incidence of intersexes was 0.3% and none were from Saanen, Toggenberg and Gems farbige breeds. The defect may be due to genetic factors linked with polled conditions. Further analysis was made of 2224 kids of 1540 goats during 1954-1955 and results obtained were similar. There was a preponderance of males among single births, multiple births and genetic sex of intersexes. The sex ratio was influenced by sires in Toggenberg and Saanen breeds but not by age of dam, number of pregnancy, year or time of service.

Van Heerden (1963) investigated the large scale abortions in Angora goats in South Africa. Angora goats moving with Boer goats and sheep aborted in high proportion though the latter did not. Frequency of abortions tended to increase with increasing levels of Angora blood. Infective and nutritional causes were excluded. It was revealed that aborting goats showed marked regression of corpus luteum of pregnancy. There was reduction in the number of acidophilic cells of anterior pituitary gland which secreted luteotropic hormone. Simultaneously, a greater activity of basophilic cells secreting follicular stimulating hormone was also noticed. The increased levels of this hormone lead to follicular development and oestrus at the time of abortion. These abortions were thus concluded to be due to hereditary factors which segregated as a result of continuous inbreeding over a period of 60 years. Van Heerden (1964) continued his studies on abortions in Angora Goats on nine farms and reported that when aborting and sterile females and sires with pedigree of aborting ancestors were culled, the incidence of abortions declined from 13 to 66% in different herds to only 2 to 10% over a period of three or four years. On the farms where no such practice was adopted, the incidence was not markedly altered.

Van Dieton (1964 b), Woodward and Clark (1959) considered that stillbirths may be genetically influenced. Colemanares (1963) reported that in Columbia, 620 of 936 White Leghorn chicks hatched were males. Hatchability of 2900 eggs from that flock was only 60%. This low figure was due to embryonic mortality probably caused by a sex linked lethal gene—'Lethal white'.

Sheridan (1964) reported from abnormal sex ratio observed in the progeny of a single broiler sire and two of his sons homozygous for the rapid feathering gene that a sexlinked gene was responsible for embryonic mortality in the rapid feathering embryos. The gene *lk* is carried by males and causes death of about half the female embryos. In a flock of Gatinaise Rhode Island Red and Wyndotte breeds with hens having recessive white plumage (*cc*) and coloured hens (*Cc* or *CC*) the hatchability of eggs from matings of *Cc* males to *cc* females was 69.58% only compared to 75.03% for mating of *cc* males to *Cc* females. This reduced hatchability was attributed to maternal effect of *cc* females which caused embryonic mortality before 18th day of incubation (Merat 1964).

ment of foetal extremities Rasbech (1950) also proposed genetic basis for prolonged gestation and described two cases with gestation periods of 420 and 455 days.

Considerable breed differences have been reported in incidence of dystokia in Western breeds of cattle.

Charolais bulls are particularly responsible for increased incidence of dystokia in pregnancies initiated by them (Milk Marketing Board Report 1960-1965). Westbye and Næss (1966) reported increased incidence of dystokia in Norwegian cows crossed with Charolais bulls. It was also revealed that some bulls cause more difficulty than the others. A part of such cases of dystokia have been attributed to the recessive lethal condition of double muscling of the calves.

### **Prolonged Gestation and Dystokia**

### Post-partum Interval

Olds and Seath (1954) reported that the time of onset of heat after calving had a repeatability of 29%. It was also estimated that about 30% of the herd variation in the occurrence of first post-partum heat was due to inheritance.

Baner (1964) reported significant breed differences in the postpartum interval to first heat at N.D.R.I. Karnal. It was 106 days in Tharparkar, 119 days for Sahiwal and 91 days for Red Sindhi cows. Differences in postpartum intervals to fertile heat were however non-significant being 163 days, 153 days and 115 days respectively.

### Calving Interval

Dunbar and Henderson (1950, 1953) studied influence of Holstein Friesian bulls over the length of calving interval of their daughters and concluded that the repeatability in succeeding intervals was low, the sire differences were practically nil and heritability value was close to zero. Pfau *et al* (1953) from their studies on the Beltsville Holstein herd during 1920-1950 also reported low repeatability and heritability values for intervals between first service and conception.

Rennie (1952) studied the causes of variation in calving intervals of Holstein Friesian cows and reported that age at first calving had no influence on length of calving intervals, 10.3% of the total variance was accounted for by the age of cow, 4.8% of total and 5.1% of herd variance was accounted for by yearly environmental differences. The repeatability estimate was 0.181 when yearly effects were eliminated and when they were ignored, it was reduced to 0.175. This indicated a rather low

heritability for length of calving intervals. Zimmerman (1955) compared the performance of 163 Black Pied Lowland cows and their daughters. When cows having calving intervals longer than 430 days were separated (compared to the herd average of 396 days) this suggested hereditary control. Alim (1957) studied the environmental and hereditary influences on calving intervals in Egyptian buffaloes and found that the repeatability of different single records of 492 buffaloes with 2243 records was  $0.179 \pm 0.023$ . The prediction of future records improved when an average of two or three records was used. The heritability of calving interval single records varied from 0.130 to  $0.076 \pm 0.156$ .

Gravett (1959) reported that the average length of calving interval of 425 daughters of a bull, whose dam, granddams and sisters all had long calving intervals as 416 days compared to 371 days for 665 daughters of another bull whose ancestors and collateral relatives also had shorter intervals. Genetic control was suggested as the probable cause.

Legates (1954) from his studies on length of calving intervals among herds in North Carolina concluded from the low estimates of heritability and repeatability that the trait is little influenced by genetic make up.

Ahmed (1961) reported from his studies on Haryana herd at Izatnagar, the heritability estimate of first calving interval of  $0.221 \pm 0.205$ . Kumar (1968) investigated the genetic influence on interval to first postpartum heat, interval between first postpartum heat and conception and the calving intervals in the same herd. Only about 2.6% of the total variation in the interval to first postpartum heat in the herd could be attri-



buted to sires. The heritability estimate for first such intervals of the 161 daughters of 13 sires was  $0.10 \pm 0.17$ . The repeatability measured from 343 intervals of 198 cows was 0.18 only. Similarly only 0.9% of the total variation in the interval between first postpartum heat and conception was attributed to sire differences. The heritability estimate obtained from 160 daughters of 13 sires was  $0.04 \pm 0.15$  and the repeatability estimated from 355 intervals of 199 cows was only 0.09. The fraction of total variation in the length of calving intervals attributable to sires was only 0.02% and the heritability estimated from the first calving intervals of 162 daughters of 13 sires was  $0.0006 \pm 0.1350$ . The repeatability value measured from 325 intervals of 196 cows was 0.03. Singh and Datta (1962) reported from their studies

Swenson (1963) from his studies of 947 daughters of nine sires reported that a group of 53 daughters of one sire had significantly lower level of fertility than the others. The average service period of these daughters was 133.4 days compared to 114.7 days which was the overall average. Investigations on five other daughter groups of the same sire confirmed that his all daughters had subnormal fertility.

Majala (1964) concluded from a very exhaustive study of about 500,000 service periods of Finnish and Swedish cattle that frequency of calving intervals longer than 15 months and number of services per conception appeared to be the best measures for reproductive efficiency.

calving intervals of the animal with the optimum interval of 365 days. Tomar (1965) and Tatke and Sane (1970 b) have pointed out the inadequacy of this measure since no account is taken of the age at which the reproductive cycles commence. Tomar (1965) proposed to tag together the age at first calving and the length of calving intervals and compared it with the optimum age at first calving of 1020 days and the optimum calving interval of 365 days. Tatke and Sane (1970) have pointed out the bias introduced even in this measure as a result of the tendency for calving intervals to reduce with advancing age

until physical maturity is reached and also on account of the method of estimation itself. Hence even this measure would not be useful for early selection for efficiency of reproduction as advocated by Tomar (1965). Eswara Reddy (1969) reported a high negative genetic correlation of  $-0.785 \pm 0.19$  between age at first calving and breeding efficiency. This would indicate that a large fraction of genes responsible for early maturity are also responsible for higher breeding efficiency.

The heritability for breeding efficiency reported by various workers is presented below

## EFFECTS OF MATING SYSTEMS

### Inbreeding

Inbreeding is often incriminated as the cause of marked reduction in fertility levels. The famous Duchess strain of Shorthorn cattle was maintained at an average level of inbreeding of about 40% against the average of about 20% for the whole of the breed in consequence of which this strain developed incidence of infertility and sterility. Gregory *et al* (1945) explained sterility in this strain of Shorthorns on the basis of a sex linked autosomal recessive gene for female sterility. Swett *et al* (1949) reported from the inbreeding experiment of Grade Holsteins at Beltsville that there was a reduction in the weight of pituitary to the extent of 19.8% in the most highly inbred group. Parathyroid and pancreas glands had also reduced in weight but the pineal and adrenal glands were unaffected. In contrast, the most highly inbred group had increased weights of thyroid. This may be compared with the findings of Bane (1954) that the weights of pituitary glands of identical twins were markedly similar and variations were recorded between twin pairs. Such relationship also existed in respect of parathyroid and adrenal glands though to a lesser extent. Such work would indicate that the inherent differences in fertility levels are dependant upon the differences due to size of endocrine glands i.e. the levels of hormone production.

Eventhough, Fincher and Williams (1926) reported high incidence of genital abnormalities like White Heifer disease in Holstein Friesian cattle due to incestuous matings. Spriggs (1946) found that only in one out of five herds studied, the condition was associated with inbreeding in Shorthorn cattle.

Lagerlof (1948) described a form of hypoplasia of testes in inbred Holstein-Friesian herds.

In the Beltsville Gurnsey herd, inbred from 1913 to 1933 and in another grade Holstein herd till 1943, Swett *et al* (1949) concluded that inbreeding had no adverse effect on fertility but inbred bulls become impotent or died at an early age. Woodward and Graves (1946) reanalysed the data and felt that the number of generations over which inbreeding was carried out was more harmful than the coefficient of inbreeding. The number of services per conception increased as the level of inbreeding increased. This was largely due to one bull and when his records were eliminated, the average number of services per conception increased only from 2.00 to 2.93 with the corresponding rise in inbreeding coefficient from 0 to 50.

Gregory *et al* (1945) studied the effect of inbreeding in the California herd and compared the incidence of sterility at different levels of inbreeding. The Jersey cows with inbreeding coefficient higher than 0.03 had an overall incidence of sterility of 7.41% compared to only 3.09% i.e. less than half in cows with lesser inbreeding. Bartlett and Margolin (1944) found sire family differences in conception rate in the New Jersey herd inbred to four foundation sires. The effect in Holsteins was less marked. Loss of fertility due to inbreeding was also recorded by Akopjan (1945). However, the only solitary example of high fertility associated with inbreeding was reported by Lowe (1938).

Robertson (1954) analysed production records of 80 British Friesian heifers produced by daughter x father matings and found that inbreeding had no adverse effect on age at first calving. Ragab

and Abdelnoor (1968) studied the data of Netherlands Cattle Herdbook Association involving 85 randomly chosen sires and 2075 daughters over five generations during 1909 to 1959 and found that with each increase of 1% in level of inbreeding, the average age at first calving tended to increase by one month from the initial average of 30 months.

Harris *et al* (1960) reported highly significant differences between incrossed and inbred bulls in respect of four semen characters. 22.6% of 215 inbred bulls, 0.8% of 122 linecross bulls, and 11.5% of 96 outcross bulls were classified as unsatisfactory in overall breeding soundness. Inbreeding had a significantly adverse effect on the morphology of spermatozoa. Age at sexual maturity tended to be retarded due to inbreeding. Though the variations in levels of inbreeding were not closely associated with levels of fertility, inbreeding appeared to affect fertility within certain lines. Rendel and Venge (1961) also reported that inbred bulls tended to have reduced fertility.

Young (1965) explained that the harmful effects of inbreeding were due to uncovering the deleterious and detrimental genes causing reduced viability and fertility.

Inbreeding in swine is often reported to be a cause for delay in onset of puberty. Warnick *et al* (1951) reported that when coefficients of inbreeding reached levels of 0.27 to 0.29, there was a delay of one month in onset of puberty in purebred and crossbred Chesterwhite and Yorkshire pigs. Squires *et al* (1952) estimated that for every 10% increase in inbreeding of parenteral lines, there was a delay of about 13 days in attainment of puberty.

Rigor (1963) reported in Berkshire pigs that inbreeding of dam had a significant direct negative influence on litter size. There was a decline of 1.9 pigs per litter for every 10% rise in inbreeding. The inbreeding of the litter itself had no significant effect on litter size. Venev (1961) reported the average litter size at 2 months of age in six very closely inbred litters as 8.17 compared to 9.54 for 119 closely inbred litters, 9.44 for 599 moderately inbred litters, 9.78 for 615 slightly inbred litters and 9.18 for 124 outbred litters. Except for the very highly inbred lines, the differences were very small. Noland *et al* (1964) studied 3432 Poland Chiana piglets from 413 litters in an inbred line over a period of 22 years. Inbreeding coefficient of dam had a significant effect on the total live piglets farrowed per litter. The more highly inbred sows farrowed more piglets per litter but as the level of inbreeding rose the number of piglets alive at birth and at 56 days declined. It was concluded that increasing levels of inbreeding do not necessarily reduce productivity. Krascenko and Pocernjaev (1964) reported from their studies on Large Whites that inbred gilts had lower fertility, a slower growth rate (leading to late maturity) and higher mortality than outbreds. When inbred males were mated to unrelated females, fertility and growth rate of foetuses during gestation were not appreciably different from outbreds though the former matured earlier.

Doney (1961) reported that inbreeding in Merino Sheep had a complex effect on fertility and the inbred ewes appeared to be more variable in their response to environmental fluctuations in terms of successful pregnancies. Doney and Smith (1968) compared the reproductive performance of 41 inbred Scottish Black-

face ewes run with tested rams at normal mating time with 26 noninbred halfsibs of similar ages. From the 41 inbred ewes, 16(39%) were infertile due to several causes viz. anovulation (6 ewes), nondeveloped or abnormal ova (4 ewes) and nonviability of early implanted embryos (6 ewes). The one noninbred ewe was infertile due to non-ovulation.

Onishi (1954) reported that inbreeding increased the mean age at first egg but did not decrease the variability. Tebb (1957) reported that in the Leghorn flock of University of California, subjected to continuous selection for egg production since 1932, showed a highly significant negative regression of about one egg for every per cent rise in the coefficient of inbreeding.

Lowe and Wilson (1964) reported from a study of 13,479 White Leghorns including 6475 inbreds over period of 4 generations, that during last 6 batches, mortality of inbreds was 0.5 to 16.7% higher.

### Cross Breeding

Verley and Touchberry (1961) studied the reproductive performance of 86 first generation crossbred heifers by comparing services per conception, interval from first service to conception, gestation period, calving interval, postpartum interval to first heat and postpartum interval to first service and found a consistent though small and nonsignificant improvement as a result of cross-breeding. It was postulated that nonadditive gene action is relatively unimportant in reproductive performance. Compere (1964) on the other hand reported that crossbred Sahiwal x Ankole heifers reached maturity at 29.5 months compared to 35.4 months for their Ankole

half sisters. There was however no improvement in calving intervals, by 457.0 days and 409.2 days. Naidu and Desai (1965) pointed out that in addition to inherited factors, adaptability to local conditions play a more important role in achievement of early maturity of Holstein x Sahiwal heifers.

Wiltbank *et al* (1966) compared the performance of Hereford, Angus and Shorthorn and all types of reciprocal crosses and concluded that heterotic effects were generally important for all crosses for age at puberty and specifically in Hereford x Shorthorn crosses. Gaines *et al* (1966) found that when purebred, Aberdeen Angus, Hereford and Shorthorn cows were crossed with purebred bulls of other breeds in this group, such matings produced 6% more calves than those purebred matings (Shorthorn cows 76% v/s 89%). Turner *et al* (1968) concluded that a considerable heterosis is present in fertility and is of great economic importance. 645 cows in this study were purebred and belonged to Brahman, Brangus and Hereford breeds and 700 were crossbreds, in addition to bulls of these breeds, Charolais and Shorthorn bulls were also used. Reproductive performance was measured as number of calves born and percentage of cows bred. It was 66% in purebreds, compared to 75.6% for crossbreds. All types of crosses were superior to parenteral purebreds.

Joubert (1962) recorded that crossbreeding both by Merino and Blackhead Persian with Dorsethorns reduced the postpartum periods by almost 50% as compared to either pure Merinos or Blackhead Persian. The percentage of ewes coming to heat within five months of lambing was Merino 64%, Dorset

Horn x Merino 100%, Black head Persian 82% and Dorsethorn x Blackhead Persian 100% Fox *et al* (1966) compared the reproductive performance of Columbian and Hampshire ewes and their crosses over a period of four years. Half of each group was mated to a Southdown ram and the remaining half ewes to a Border Cheviot ram. The percentage of crossbred ewes lambing was 84% compared to 71% for purebreds and that of lambs born per ewe lambing was 1.41 and 1.31 respectively.

Donald (1963) found less incidence of stillbirths in crossbred mothers. Smith and King (1964) reported that crossbred sows were superior in litter size and had reduced piglet mortality. Skarman (1965) reported from crossbreeding of Swedish Landrace x Large White pigs that crossbreds produced 0.7 piglets more per litter. Dimov (1967) compared the performance of Bulgarian White and crossbred sows and reported that in gilts slaughtered at 74 days postmating the number of corpora lutea, embryos and embryonic weights were greater in crossbreds than purebreds. In sows that farrowed the average litter size and birthweights were also greater for crossbreds.

Onishi (1954) reported from his studies on White Leghorn Single combed birds that sexual maturity was earlier in crossbred birds and the variability was significantly smaller than in parental inbreds.

### INHERITANCE OF FERTILITY AND INFERTILITY IN THE MALE

Infertility in males is being investigated as a potent factor for losses incurred in livestock industry only since the last few decades. Awareness of genetic factors determining the levels of fertility of males was realised even later.

The investigations into genetic control of male fertility can be broadly classified as follows:

- 1 Anatomical defects of the genital organs
- 2 Disturbances in spermiogenesis and gametic sterility
- 3 Variations in sexual behaviour and mating
- 4 Variations in seminal attributes of bulls leading to differences in fertility levels

#### Anatomical defects of genital organs

Anatomical defects of the genital organs may not be ordinarily detected until onset of puberty and maturity. They may be caused as a result of hereditary predisposition. It is interesting to investigate genetic causes of such anatomical defects and try to reduce their incidence by culling the carriers.

#### A DEFECTS OF PENIS

From reports available from literature it can be said that anatomical defects in penis are rare. Arndal and Nes (1958) reported from their studies on five Norwegian Landrace boars that all of them had their glans penis attached to prepuce. Another litter of four females and three males had two males with similar defect. As the five affected animals belonged to the same line it was suggested that the defect might be inherited. Wierzbowski (1959) concluded from available information that the incidence of hereditary impotence in boars was increasing. Dora (1956) observed in 15 bulls an anatomical abnormality in volving retroversion of the end of penis during erection which prevented a successful copulation. The abnormality apparently of genetic origin in nature is capable of surgical correction and hence

should be so corrected only for production of beef stock for slaughter.

#### B. DEVELOPMENTAL ABNORMALITIES OF STRUCTURES ORIGINATING FROM THE WOLFFIAN DUCT

Ashdown (1958) described the anatomical abnormalities seen in the cases of segmental aplasia of the Wolffian ducts in British Friesian bulls. One was the offspring of a son x dam mating and the condition was bilateral. In the other bull, only the right epididymis, vas deferens and ampullae were affected and the ejaculate had good quality. Affected testes invariably showed degenerative changes. It was mentioned that identical cases have been reported from Denmark also. Ashdown (1962) believed that cases of persistence of penile frenulum in bulls which makes service difficult, was congenital.

Blom and Christensen (1960) investigated during 1955-1960, 2,000 Black Pied Danish and 2,015 Red Danish bulls of which 34 (2.1%) and 27 (3.9%) respectively had normal development but there was a lack of development of segments of epididymis, the ductus deferens and the vesicular glands. In most cases, this defect was unilateral and caused spermiostasis, bringing about a swelling in the head of epididymis on the affected side. They also mentioned that because a similar bilateral condition found in hilly goats was known to be hereditary, similar cases found in bulls and rams must also be so. Though a definite proof could not be given, some recessive factor was thought to be responsible. Schonherr (1954) found from a study of 1436 white German improved goats that 280 (19.5%) of them were infertile and of these 65.5% suffered from sperm congestion. Lohle

and Herman (1955) reported that out of 3001 bucks presented for licensing during 1950-54, 11.4% had congestion of seminal vesicles and 5.6% had undeveloped testes. It was suggested that these conditions are inherited.

#### C. ABNORMALITIES OF PREPUCE

Donaldson and Aubrey (1960) reported from a study of 25 cases of posthitis and prolapse of prepuce leading to sterility in bulls aged 1½ to 9 years of age, that 19 were Santa Gertrudis, two Santa Gertrudis crosses, three Zebu crosses and one Hereford. It was suggested that breeders should select against this trait with possible genetic involvement. Long (1969) observed from a study of 254 bulls of 13 breeds varying in age from 1 to 13½ years at six A.I. centres that a relationship appeared to exist between polling and eversion of prepuce of the 33 naturally polled bulls, 85% everted compared to 1.4% only amongst the 211 horned bulls. No case was recorded in 96 horned Friesian bulls but in a herd of polled Friesians, six out of seven polled bulls everted the prepuce.

#### Testes

##### D. CRYPTORCHIDISM

Cryptorchidism condition, unilateral or bilateral, has been met with in all the species of farm animals and this is claimed to be having hereditary bias.

##### E. GONADAL HYPOPLASIA

Hypoplasia of testes, whether unilateral or bilateral has been reported in all the farm animals and the cause has been attributed to a recessive gene with incomplete penetrance.

## F. PSEUDO HERMAPHRODITISM

This condition is more common in males than in females. Some of the strains of pigs are more prone to intersexuality than others, suggesting probable interplay of genetic factors. Baker (1925) reported that in New Hybrides, a purposeful selection for a high proportion of intersexes has been possible because it is of hereditary nature. Hafez and Jainuddin (1966) concluded that though intersexuality may be due to a single recessive gene limited to male sex, possibility of additive genetic effect can not be over ruled. Albertschen (1951) and Mohr (1953) reported occurrence of different forms of hermaphroditism in the same litter. Nilsson (1962) incriminated inbreeding due to widespread use of A.I. in pigs in Sweden for the sharp increase in incidence of hermaphrodite pigs. Hamari (1965) reported an incidence of 0.83% intersexes in 1233 pigs in three herds. One hermaphrodite

cultures of 25 intersexes examined by them were all of XX type.

## Bulls

Haq (1949) stated that bulls of European breeds will mount cows in heat or otherwise. They also mount on other bulls. In contrast Zebu bulls have a characteristically wellmarked sexual sluggishness (Anderson, 1948, 1949) and will mount only cows that are in full oestrus. Smith (1951) and Edwards (1955) reported that Shorthorn and Guernsey bulls react more slowly than Friesian bulls. Stimulation of bulls improved the conception rate of Friesian bulls more than that of Shorthorn or Guernsey bulls. Smith (1951) found that Hereford and North Devon bulls are most docile. James (1950) concluded that the pattern of slow serving is inherited and cannot be modified even with drastic experimental methods.



was significantly higher (25.05% compared to only 8.4% for normal bulls). Eriksson (1949) postulated that a proportion of cases of inability to copulate in bulls was due to an inherited hormonal disturbance. In a later study, Eriksson (1950) reported from an analysis of 3266 Swedish Red and White bulls that hereditary factors do contribute to reproductive disorders such as slow serving nature and infertility. From the study on wastage in insured bulls, it was found that bulls of some families of Swedish Red and White breed tend to be supranormal and others subnormal. It was also concluded that disturbances in fertilizing ability are due to an interaction between hereditary factors and environmental factors.

From an extensive study of several thousand insured, Swedish Friesian, Swedish Red and White and Swedish Highland bulls, Lagerlof (1948, 1950) found great differences between these breeds in frequency of serving impotence. The information is tabulated below (Table 77).

Lagerlof (1948) believed that endocrine factors associated with feminine type may be a contributing cause for weak sex drive. Lagerlof (1950) cautioned the breeders against selection of feminine type of bulls lacking in secondary sexual characters. Such bulls,

though fertile, are usually poor servers and this is aggravated under adverse environmental conditions. De-Groot and Norman (1946) recorded this condition in 22 Friesian bulls and considered it to be due to a recessive factor.

Hanson and Bane (1950) felt that hereditary factors seem to play a decisive role as regards sex drive. Bane (1954) from his studies on twin bulls reported that differences between pairs were greater than those within pairs, which lead to the conclusion of a hereditary control of sex drive. An example is cited of a twin pair which suffered from affections of joints and served normally all their life, whileas another pair free from such defect became impotent at 3½ years of age only. James (1950), Olson and Peterson (1951) and Bane (1954) have all suggested that variations in serving ability are largely controlled by the genotype of the bulls. Olson and Peterson (1951) found that all the members of a set of monozygotic triplets were slow servers, McMeekan (1952) studied 10 to 15 pairs of identical twins over five years and showed that the sexual performance of bulls is extremely hard to be influenced by environmental control which indirectly means that it must largely be determined by genotype.

Trautmeim (1954) studied records of 393 spotted Mountain bulls and found

Table 77  
FREQUENCY OF SERVING INABILITY

Breed Year	Swedish Red & White (SRB) 1928-44, 45-49		Swedish Lowland 1928-44, 45-49		Swedish Polled Breed (SKB) 1928-44 45-49	
Impotentia cocundi	34%	41%	30%	45%	68%	72%
Impotentia generandi	23%	20%	18%	12%	4.8%	3.0%

berg (1967) presented a genealogy of Black Pied Lowland cattle, several members of which were affected with impotentia coeundi. The condition was first detected when a bull which gave collections in an artificial vagina could not serve cows naturally. The condition was postulated to be transmitted as an autosomal dominant gene. Females did not show any deviations in sex characters.

Inherited defects of the feet, joints and bones etc. and especially those of hindlimbs may reduce the serving ability of bulls. Several of such defects have been known to have hereditary bias. Neilsen (1950) described a hereditary form of lameness in Red Danish bull calves. Gottwald (1953) described cases of interdigital chondroma resulting from a dominant mode of inheritance. Van Drimmelen (1942) reported that 8% of the progeny of a Friesian bull in South Africa, suffered from lameness and believed the condition to be inherited. Syndactylism reduces the mobility of affected animals and has been reported to have a hereditary bias in Hariana (Singh and Tandon, 1942; Singh and Bhattacharya, 1959). This defect was found to be due to autosomal recessive gene; Holstein Friesian (Eldridge *et al*, 1951) and in Nipponese improved cattle (Motahishii, 1951). Van Schaik (1956) observed in Dutch Friesian cattle, hereditary defects of hooves and crooked hindlimbs in which excessive weight was brought to be borne on heels and which ultimately lead to unsoundness.

Hansen (1964) studied 49 sons of 48 cows on 38 farms all sired by a single Red Danish bull of which 65% were affected with arthritis serosa tarsi. At three years of age the incidence was 71% and at 4 years as high as 81%. Sus-

ceptibility to this condition disturbing normal mating behaviour was believed to be inherited.

### Horse

Vander Plassche (1955) observed that in spite of normal mounting, 19 stallions of Belgian breed failed to ejaculate. In a third of these, the defect was noticed in the first breeding season and in the remaining, after serving normally, in one or more seasons. This purely functional anomaly was thought to be due to recessive genetic factors getting expressed due to adverse environmental conditions.

### Sheep

Hulet *et al* (1962) studied mating behaviour of 32 rams, each allotted 18 to 32 ewes in a pen. Variations in meteorological conditions (except for amount of sunlight) or in type of artificial illumination had no apparent effect on mating behaviour. Most important factors were individual differences and number of ewes on heat. When the latter was held constant differences between rams were highly significant. Ramboulet rams were most active breeders followed in order by Taighese and Columbia rams.

### Pigs

Schilling (1962) reported from an in-breeding experiment with brother sister mating in swine over four generations that three boars of the fourth generation showed impotency. Histological examination of testes revealed no reduction in hormone producing interstitial tissue and no other histological abnormalities. Spermiogenesis was also normal.

### Poultry

As regards inheritance of fertility and infertility, considerable work has been done in Poultry.

Mc Daniel and Craig (1959) studied reproductive behaviour of 120 White Leghorn cockrels and its relationship to semen characteristics and fertility. Significant associations were found between social aggressiveness score, sexual effectiveness score (number of successful matings in 10 minutes) and crouches elicited from hens. Sexual effectiveness was significantly associated with fertility.

A very interesting series of experiments revealed that reduced fertility of rose combed cockrels was ultimately traced to reduced sex desire. Crawford and Smyth Jr. (1964 a) investigated the causes of reduced fertility of homozygous rose combed males in poultry. When RR, Rr and rr males were mated to rr hens, no significant differences in the ratio of these three genotypes was found, indicating that there was no deficiency of progeny of one genotype or sex. It was observed from Rr X rr mating that neither the fertility of sperm cells carrying the R gene nor the duration of fertilizing capacity was reduced. RR males produce only sperm cells with R gene which means that the deleterious effect of R gene is not within the spermatozoa but of the RR host body producing the spermatozoa. It was postulated that the R gene acts as dominant in its expression on comb shape but as a recessive its effect is on fertility. Crawford (1964) conducted two trials in which 11 walnutcombed males (5 RRPP and 6 RrPp) were used to inseminate white leghorn (rrpp) hens. In the first trials, the egg fertility and the fertility duration of matings by RRPP males averaged 61.75% and 8.15 days compared to 81.08% and 12.02 days by mating with RrPp males. The corresponding figures in the second trial were 76.80% and 10.23 days compared to

83.24% and 14.07 days. These differences were highly significant. Thus the adverse effect of homozygosity at the rose comb locus in males was not modified by the gene for pea comb. Crawford and Smyth Jr. (1964 b) studied the effect of R and r alleles in the Ottawa Meat Control strain of poultry under conditions of natural mating and A.I. Low fertility was marked only from males homozygous for rose comb (RR) heterozygotes (Rr) and single combed (rr) males reproduced normally. Females of all the three genotypes had normal fertility. Examination of incubated eggs showed no evidence for increased embryonic mortality. When females of genotype were inseminated with semen of males of all three genotypes there were 30% more sterile mating with RR than Rr or rr mating. The interval between insemination and production of the last fertile eggs was lower with RR males than those of other genotypes. Crawford and Smyth Jr. (1961 c) inseminated hens with RR spermatozoa in rr seminal plasma. RR spermatozoa separated and resuspended in RR. Similar treatment was given to rr spermatozoa also. There was no indication that rr seminal plasma affected the fertility of RR spermatozoa or vice-versa. No significant differences existed between RR, Rr and rr males in semen volume, percentage of dead spermatozoa and abnormal spermatozoa, motility, sperm concentration and methylene blue reduction time.

Crawford and Smyth Jr (1965) studied five homozygous Rose Comb (RR), 27 heterozygous rose comb (Rr) and single combed (rr) cockrels. Copulations, female crouches and three forms of male display were recorded. Crouching of female was significantly less in females exposed to RR cockrels than

when they were mated to Rr or rr males. It is suggested that the reduced fertility in RR males in natural mating might be due to their low mating frequency. Tindell and Arze (1965) reported from an experiment on 300 Athens Canadian foundation flock of poultry for selection of mates for high and low mating ability that they measured number of matings completed in four and ten minute periods by 28 week old cockrels. The selection was carried out for three generations. The mating ability of the high and low groups was 7.63 and 3.45 in the first generation and 13.63 and 5.57 in the second generation. Age at sexual maturity was significantly greater at 14.79 weeks in the high group compared to 12.89 weeks for the low mating group.

### CHROMOSOMAL ANOMALIES LEADING TO INFERTILITY AND STERILITY

#### Cattle

Leuchtenberger and Schroader (1955) reported from a study of a dwarf bull in a pure Hereford herd that the amount of DNA in its somatic cells was only slightly more than half the amount present in normal bulls. Similarly, the spermatozoa in the epididymis and seminal vesicles had half the normal haploid DNA content. Spermatozoa within the seminiferous tubules were however of two types i.e. those with normal haploid content and those with reduced content. The effect on fertility is not mentioned.

Peters and Newbound (1957) investigated the causes of male sterility in crosses of American bison and beef cattle, from their studies on 7 bisons, 11 Hereford and 11 Cattalo (1/8 to 3/8 bison) yearling bulls. The mean testicular temperatures were  $93.9 \pm 4.0^\circ\text{F}$ ,

$91.0 \pm 3.7^\circ\text{F}$  and  $86.8 \pm 5.5^\circ\text{F}$ , respectively. The difference between Herefords and Cattalos was almost significant. The mean body weight and testis weight (in brackets) were 596.2 lb. (53.3 g), 770.6 lb. (201.3 g) and 776.4 lb. (89.8 g.) One cattalo was fertile, 3 had a low degree of fertility and 7 were sterile.

Knudsen (1958, 1961 a, b) described two types of sterility in bulls, both presumed to be of genetic origin.

#### A. STICKY CHROMOSOMES

Knudsen (1961 a) reported from his cytological and morphological studies on ejaculates of five Holstein Friesian bulls and one Red Danish bull having slight bilateral testicular hypoplasia, that all the bulls had good libido. The chromosomes of the dividing spermatocytes instead of passing in two daughter cells, tended to stick together. This resulted in chaos in succeeding meiotic divisions. The ejaculates were characterised by aspermia and oligospermia. Histological examination of the testes showed that pyknotic nuclei were distributed throughout the seminiferous tubules and the chromosomes tended to stick together. Majority of the cells showed karyopyknosis or karyolysis. The semen was thin and watery when the samples were centrifuged, the sediment contained mostly pyknotic nuclei and few hyperchromatic spermlike structures.

#### B. MULTIPLE SPINDLE FORMATION

The other type of abnormality was observed in three Swedish Lowland bulls aged between  $1\frac{1}{2}$  to 3 years, culled on account of sterility resulting from poor semen quality. The bulls exhibited unusually strong libido but had a moderate testicular hypoplasia. Cytogenetic stu-

dies revealed that the spermiogenesis was arrested due to multiple spindle formation. The chromosomes divided without the succeeding division of cytoplasm. These bulls produced watery ejaculates containing giant cells, pyknotic nuclei and few spermatozoa. Two of these bulls had the same sire and all three had the same paternal grand dam. As possibility of toxic effects could be eliminated, it seemed possible that the defect could be inherited.

Both the above types of abnormalities could be diagnosed with ordinary microscope.

Knudsen (1961 b) from his electron microscopic studies of spermatozoa of bulls which were excellent, according to conventional methods of assessment and yet had low fertility reported that four of these bulls were inversion heterozygotes and three others were translocation heterozygotes. During meiosis, in the former group, the chromosomes of the inversion pair formed a loop. When crossing over took place within the loop, one of the resulting chromosomes had two centromeres and the other lacked a centromere. The latter lacks orientation during cell division and may pass in either of the two daughter cells. The former formed a bridge between two groups of chromosomes and after it gave way, two unbalanced daughter cells were formed. Such types of spermatozoa may be capable of fertilization of ova but the zygotes formed have questionable viability.

The male hybrids of the first and second (backcross) generations to Yak were however sterile though they exhibited normal sexual behaviour. Spermatogenesis did not proceed beyond the spermatocyte stage. Normal spermatogenesis occurred in the second backcross generation bulls. There appeared to be a genetic relationship between polled condition and spermatogenesis, since the polled bulls of second generation did not show any sign of spermatogenesis.

Trujillo *et al* (1969) reported spermatogenesis in a chromosomally verified male hinny. Spermatogonia of large size (indicating tetraploidy) actively engaged in mitosis were observed in the left testis. The secondary spermatocytes appeared to contain four nuclei each. Wellformed spermatozoa were occasionally found in the right testis but they were immotile and tended to clump together. Some had either two heads or two tails. Since the spermheads and sperm tails approximated the mean dimensions of the two parental species, they were considered to be haploid and not diploid. No conception occurred in spite of repeated matings to fertile mates indicating sterility.

to 86 kg. This condition corresponds to the Turner's syndrome in man.

Ishihara (1955) stated that in none of the male tortoiseshell cats studied by him, spermatogenesis could be observed in the testes. Sertoli cells were present but most of the spermatogonia showed signs of pyknotic degeneration. A few abnormal primary spermatocytes were observed in one cat only. Ishihara (1956) from his cytological studies on six male tortoiseshell cats reported that in one, spermatogenesis was absent, in two, a few spermatogonia were found, in the fourth cat, secondary spermatocytes were found and in the remaining two, spermatogenesis was apparently normal. In these apparently normal tomcats, the diploid chromosome number in the spermatogonia and spermatocytes was 38 including the two sex chromosomes i.e. the same compliment as in normal male cats. Komai and Ishihara (1956) collected records of a total of 55 male tortoise shell cats and actually examined 24 of them. The colour pattern of males was similar to female tortoise shell cats. However such males tended to be timid, had smaller sized testes and lacked sexual behaviour even in breeding season. The testes of four sterile tomcats were examined histologically and no spermatozoa were detected. Degenerative changes were seen in germ cells. Three apparently fertile males had histologically normal testes with 38 chromosomes as in normal males. It was suggested that the gene 'O' (orange colour) is only partially sexlinked and can be transferred from X to Y chromosome by crossing and of the pairing segments. It was assumed that a gene or gene complex situated on the Y chromosomes, governing fertility is also transferred along with O from X to Y chromosome. If on the rare occa-

sions, the latter did not cross over, fertility would be normal.

Thuline (1964) suggested that male tortoise shell, Calico cats have an XXY chromosome constitution. The presence of 2 X chromosomes allows both black and yellow as in females, while the Y determines maleness. Such cats are sterile. Few such tomcats had 39 chromosomes instead of normal 38. In fibroblast cultures of one tortoise shell male, a normal 38 (XX) cell line and a 57 triploid (XXY) cell line were observed. This was regarded as a chimera resulting from fertilization with two different sperms. Another unusual cat had a cryptorchid testis on one side and an ovary on the other. The coat was white and it had one yellow and one blue eye. The chromosome count was normal but some cells were XX and other XY.

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# Chapter 51

## Nutrition in relation to Fertility

### PLANE OF NUTRITION

#### Cattle

##### FEMALE

Asdell (1955) stated that underfeeding of heifers delays the onset of first oestrus. Joubert (1954) reported that late puberty (by 9.6 months) was observed in heifer calves raised on pastures only during the period of winter depression. The plane of nutrition during the growth period has a marked effect on the age of puberty and on the occurrence of first oestrus. Once the puberty is established with proper body size and regular oestrous cycles commence, the effect of plane of nutrition whether high or low is not so intense.

Sorensen *et al* (1959) observed variations in the average age at first oestrus in heifers reared on high, medium and low levels of feeding as 37.1, 41.1 and 70.0 weeks respectively. Similar findings on heifers raised on different planes of nutrition have been observed by Reid *et al* (1951). Patel *et al* (1956) reported similar observations in Kankrej cattle.

during late pregnancy as per NRC standards. Zimmerman *et al* (1961) found that it is not the restricted intake of protein but the low energy levels that are responsible for delay in the onset of post partum oestrus.

Mahadevan (1962) stated that the practice of flushing and steaming up to increase fertility and production is in vogue but such practices may lead to reproductive disturbances due to high plane of nutrition.

Rangiah (1971) claimed early post partum heat and conception (within 90 days) in Gir cows maintained on high plane of nutrition in the 1st trimester of pregnancy, compared to the delayed period of 4 to 6 months in the same herd.

attain puberty much later compared to the adequately fed ones. In an experiment on Holstein bull calves with high plane of nutrition, motile spermatozoa were observed as early as 37 weeks compared to 51 weeks in the group maintained on low level. When the semen production of these bulls was studied at 78-80 weeks, the high level group had about double the sperm concentration than that of low level group with their accessory organs tended to be small indicating a probable failure of testosterone secretion. In an experiment where only the energy intake was reduced but other nutrients fairly kept on a constant level, Asdell (1955) observed that there was delay in spermatogenesis and reduction in the number of spermatozoa. Flipse *et al* (1953) reared 4 groups of Holstein bulls on different levels of nutrition. One was maintained on a low level (70%), another on normal (100%) and two on high levels (115% and 130%). The mean ages when motile spermatozoa were first observed in an ejaculate were at 66, 46, 41 and 43 weeks respectively. There was no difference in the volume of ejaculate, pH and percentage of live spermatozoa in the different groups. The different levels of nutrition caused considerable variations in body weight by about 96 weeks of age, but the skeletal development was least affected.

VanDemark *et al* (1964) observed differences in the age and weight at puberty. Underfeeding delayed the production of optimum levels of semen by about 8 months. The weight of the carcass and weights of the testes, epididymis, Cowper's gland, pituitary and adrenal gland except the seminal vesicles were always less for the restricted group even after placing them back on normal rations.

However, the animals regained in body weight. Effects of reversal of feeding levels in both the groups were slight except that in the lower group regeneration was slower.

Studies made by Branton *et al* (1947, a & b) indicate that level of protein to the extent of 12% in the feed was sufficient for dairy bulls. Feeding bulls with 20% protein resulted in a higher concentration of sperm but lower volume and total sperm per ejaculate. Meacham *et al* (1963) observed that as against normal protein level of 14% in feeds, young bulls which were fed less than 2% protein had low semen volume and the total sperm ejaculate decreased considerably.

Mann (1964) studied the nutritional effects on the functions of accessory glands and found that nutritional effects on these glands may precede changes in spermatogenic function. Examination of the semen samples revealed that androgenic function as indicated by the appearance of fructose and citric acid in the semen, was retarded in underfed calves upto 4 months or so. The appearance of the spermatozoa was also delayed. It is evident from the studies of Mann (1964) that the age of puberty in young bulls was affected by nutrition, sperm production was depressed and it is difficult to overcome such a condition, even after subsequent improvement in feeding.

It has been conclusively proved that adult bulls are less susceptible than young ones to effects of underfeeding except reduction in fructose and citric acid levels in semen (Quicke *et al*, 1950; Graham and Fredrick, 1957 and Shirley *et al*, 1963). Various functions



of the reproductive organs may however be set right by adequate feeding

## Sheep

### FEMALE

A review of literature shows that evidence on the effect of the plane of nutrition maintained prior to and during breeding season on the onset of oestrus and the length of oestrous cycle is not very clear. Hafez (1952) observed that underfeeding did not delay the onset of breeding season in Suffolk ewes yearlings and adults placed on sub maintenance feeding for about 3 months prior to the expected onset of breeding season and they came into heat at the normal time even in spite of the loss of about 45% of their initial weight. Allen and Lamming (1961) observed significant lengthening of the oestrous cycle and increase in the incidence of silent heats in the ewes on sub maintenance diet. They observed high ovulation rate in ewes. Smith (1965) however found that there was a fairly marked delay in oestrus with decreased intensity in ewes placed on sub maintenance feeding during winter and spring. Gerring (1954) observed that there is no evidence that flushing in ewes leads to early commencement of breeding season. Wallace (1954) observed that ewes which were flushed had significantly shorter cycles than unflushed ewes.

Hafez (1952) found that a number of silent heats were on the increase in ewes on sub maintenance diets and feeding a super maintenance diet resulted in quick recovery for setting up normal cycles.

Wallace (1954) and El Sheikh *et al* (1955) observed that ovulation rate is on the increase in ewes fed at a high level prior to mating. Foote *et al* (1959)

concluded that the body weight at the time of breeding in yearling ewes was more important in determining rate of ovulation than changes in the nutritional status. Body weight and plane of nutrition affect the rate of ovulation in ewes. Flushing for 4 weeks or more has a beneficial effect on the rate of ovulation.

### Effect on Litter size

Increase in the lambing rate in flushed ewes has been reported by Vitti (1951) and Gerring (1954). Evidence has been presented by Wallace (1961) and Coop (1964-66) that higher live weight in ewes is associated with higher twinning rate and less barren ewes. Coop (1964-66) claims that flushing has a specific effect i.e. in ewes of same live weight and conception rate those gaining weight will have a higher percentage of twins than those maintaining steady live weight. Hulet *et al* (1962) and Coop (1964) have concluded that the period immediately before mating was the critical one. McArthur (1963) reported superior results by flushing on grass as compared to those on cereals. It appears from work of Williams (1951) and Hulet *et al* (1962) that young ewes may not respond to flushing with the same intensity as the older ones.

Klosterman *et al* (1951) found no significant differences on number of lambs born and number of barren ewes by placing ewes on different levels of proteins during flushing, breeding and lactations. Glen and Whiting (1952) obtained similar results. It appears from the above work that probably a level of 10% of dietary protein is considered adequate even for ewes bearing twins. It can be inferred from the work reported that in a ruminant like sheep the source

of protein or nitrogen has little effect on reproductive performance (Klosterman *et al*, 1953; Slen and Whiting, 1955).

Davenport (1967) reported work in Border Leicester X Merino ewes divided in two equal groups, one fed on oat pasture and another on clover pasture. In the first year 84.2% of the mated ewes in oat group lambbed as against 97% in the clover group. However, in the fifth year 87% of the oat group lambbed, versus 46% only of the clover group. The lamb mortality was 18% in oat group and 39% in the clover group. The lambing percentage increased steadily from year to year in ewes on oats and reached 124 in the final year versus 66 in ewes on clover in which it showed a gradual decline. The rate of twinning was significantly lower in the clover group.

Accurate information on the effect of different levels of feeding on rams is not very precise. Hulet *et al* (1956) reported no significant effect on fertility in rams fed only on roughages or a mixture of roughages and grains. Salamon (1964) fed rams a high and low plane of nutrition and did not observe any significant difference in libido, but sperm density, volume per ejaculate and motility were higher in the high plane group.

It can be inferred from the work of Warnick *et al* (1961) that protein requirements for the reproductive processes in the ram appears to be inconsequential and whatever necessary is readily made available from the tissue of the ram or by supplementing urea as the only source of nitrogen which was found to be as satisfactory as soyabean protein.

## Swine

### FEMALE

Duncan and Lodge (1960) reported the mean age at puberty in swine as 200 days, but with marked variations with breed, environment and nutrition. Robertson *et al* (1951) failed to find significant differences due to feeding on the age at puberty but Burger (1952) observed that the underfed gilts average 235 days and 118 lb. of body weight at the first oestrous compared to 188 days and 194 lb. of body weights for the controls which were fed adlib. Gosset and Sorensen (1959) observed that the weights at puberty in gilts were 186 and 166 lb. respectively on high and low energy diet. However, their observations show that the age of puberty on the high energy diet was 214 days and on the low energy diet 206 days. The difference is not significant. Sorensen *et al* (1961) observed no difference in age at puberty between groups of gilts fed at different levels. Haines *et al* (1959) observed that gilts on high energy diets reached puberty significantly earlier at 197 days as compared to 217 days on low level and corresponding weights were 196 and 160 lb. From the above review it can be inferred that a moderate level of underfeeding has little effect on age at puberty, unless otherwise, when it is severe enough to lower the bodyweight.

Heap (1966) however, in his experiments on swine observed highly significant correlation between the body weights of the sow and ovulation rate. This supports the view that the nutritional status at the time of the oestrus has an influence on the rate of ovulation. As in the sheep, it appears that increase in the feeding standard for about two weeks prior to oestrous period may pro-

duce beneficial results. Kvasnitsky (1956) has cautioned that very high feeding level has no beneficial effect but it may likely to affect adversely. Tassell (1967) in his review has mentioned that there is growing evidence to show that a high plane of feeding immediately after mating adversely affects embryo survival. O'Grady (1963) found that 3 to 6 lb. feed daily given to gilts and sows has little effect on the size of the litter (average 10.1 and 10.3). Lodge *et al* (1966) made similar observations. Adams *et al* (1960) reported that gilts if given maize only, failed to come into oestrus. Ovulation rates were greatest on the 32% protein (Maize + Soyabean meal) and the 15% protein (Maize + Sesame + Lysine) diet. In the group receiving maize only, the weight of the anterior pituitary was the least. Fowler and

0.06% ether extract. In both male and female the sexual maturity was delayed and weights of testes and ovaries were reduced compared to their weights in those receiving 5% lard.

#### MALF

The development of the seminiferous tubules was observed to have been retarded as a result of underfeeding in boars (Niwa, 1951). The first appearance of spermatozoa was found to have been delayed by about 30 days and even at 10 months of age, certain boars had none. Spermatozoa from the cauda epididymis of underfed boars were inactive at 5 months of age, at 7 months however, they were similar to those of normal boars. It is observed that in boars, age at puberty is not affected significantly by different levels of under

of fat in the ration. Weights of testes were found to have been reduced.

## VITAMINS IN RELATION TO FERTILITY

### Vitamin A

#### CATTLE

Hart and Gilbert (1933) observed that cows grazing on dry range for extensive period gave birth to weak or dead calves and subsequent to parturition did not exhibit oestrus until they received greens.

Madsen and Davis (1949) observed that fertility was low in cows receiving 30, 45 and 60 mg. levels of Vitamin A and those receiving 90 mg. or more did show normal efficiency. However, the intensity of symptoms was dependant on the various levels of intake. Byers *et al* (1956) found that the second generation cows raised on a suboptimal carotene diet 50 mg. did not show satisfactory fertility even when the level of carotene was raised to 100 mg./Kg. body weight daily. Typical lesions of carotene deficiency such as hydrocephalus, blindness and weakness at birth were observed in the third generation calves, even the calves which appeared normal at birth but which were subjected to pre and postnatal deficiency showed pathological lesions of optic nerve, pituitary and adrenal glands. It appears that Vitamin A requirements for production are higher as compared to the needs for the prevention of night blindness. It is evident from the work of Madsen and Davis (1949) that serum levels of Vitamin A of 18 mg/100 ml. or less are not conducive for normal reproduction. It is observed from the work of Hodgson *et al* (1946), Erb *et al* (1947), Madsen *et al* (1948) and Byers *et al* (1956), that cystic

condition of the pituitary gland appears to be a constant feature of Vitamin A deficiency. Cystic condition may interfere with the production of gonadotrophin. Mahadevan (1962) has shown that in mature cattle, Vitamin A deficiency can be cured by administration of Vitamin A, but in animals in which deficiency has occurred before maturity, it may result in irreversible damage such as cystic condition of pituitary and degenerative changes. In most of the affected animals major part of the anterior lobe of the pituitary may be involved. Deficiency in mature females may result in abortions during the last third of gestation period, with retention of foetal membranes. Mahadevan (1959) on his investigations on subfertile Haryana heifers found that levels of Vitamin A are significantly lower in the case of heifers not coming to heat, than in the normal ones, though, the values for calcium, phosphorus, copper and carotene in the blood of animals in both the groups were practically the same. Mahadevan (1962) conducted investigations on groups of Haryana heifers in which there was delayed sexual maturity, even though they were between 4 to 5 years old. He divided the lot in two groups. The blood picture for calcium, phosphorus, carotene and vitamin A level before treatment was the same for all and including the grazing and managemental conditions. Experimental lot injected with 10 intra-muscular injections of Vitamin A acetate, 1,00,000 i.u. each at 3 days interval, came on heat on the 5th, 18th and 97th days after the commencement of Vitamin A therapy. They were inseminated and conceived, while the control group did not come in heat during the experimental period nor thereafter for about a year. It therefore, appears that the low level of Vitamin A in blood seems

to be an important factor for impaired reproduction.

Deficiency of Vitamin A in bulls delays sexual maturity. Hodgson *et al* (1946) reported that bulls developing Vitamin A deficiency before puberty failed to breed. From a review of studies, 50 mg/Kg. body weight, carotene appears to be adequate for the performance of reproductive functions in the bulls. Less quantities fed for a prolonged duration may result in marked increase in per cent of abnormal sperms and progressive decline in motility. The ill effects of the deficiency may be corrected by carotene supplementation, even though the damage to testicular tissue may be of longer duration as that of 10 to 20 months

nonviable lambs. Guilbert *et al* (1937) reported night blindness in sheep at about lambing time due to A Vitaminosis. Lambs born, had no Vitamin A stored in their liver. It was further observed that even 3 times the dose of Cod liver oil which was sufficient to overcome night blindness was not adequate for producing viable lambs. Liver normally acts as a store house for Vitamin A and no ill effects may be observed due to deficiency if it is of short duration, but in case of prolonged deficiency, a striking effect is observed by a decrease in the viability of lambs.

dent that Vitamin A deficiency may lead to a reduction in viability of piglets and also to malformation usually of the soft tissues.

### Vitamin B Complex

Miller *et al* (1953) found that it is advisable to supply at least 1.25 mg. Riboflavin/lb. feed to ensure that the litters have good viability. Lesser intake have been incriminated for loss of appetite of dams and death of piglets.

Ullrey *et al* (1955) maintained sows on 4 levels of pantothenic acid and found that gilts receiving 5.9 mg/Kg. conceived but farrowed piglets which exhibited locomotor incoordination and diarrhoea; those receiving 12.5 mg/Kg. or more did not differ in gestation and lactation performance. Devey and Stevenson (1963) from similar experiments concluded that second generation gilts receiving lower levels of this Vitamin had significantly fewer young and amongst those born, muscular incoordination was seen. It is concluded that 5.4 mg./lb. of feed is the minimum requirement for litter of normal numbers and viability.

Vitamin B<sub>12</sub> is regarded as the Animal Protein Factor and is considered essential for the various physiological requirements including reproduction. Moustgaard (1952) observed that four weeks after mating, gilts receiving all vegetable diet supplemented with Vitamin B<sub>12</sub> had more live embryos than the controls. Although the weights of the embryos did not differ between the groups, sows not receiving the Vitamin B<sub>12</sub> supplement produced fewer piglets per litter and the viability of those was lower than the piglets of gilts receiving Vitamin B<sub>12</sub>. Anderson and Hogan (1950) observed that gilts receiving Vitamin B<sub>12</sub> supplements could rear all

their liveborn pigs where as, the group not receiving the supplement lost about 13% of their piglets. It is seen from their work that shortage of Vitamin B<sub>12</sub> during pregnancy reduced the litter size and decreased viability of those born.

### Vitamin C

#### CATTLE

Mahadevan (1962) has explained the vital role of Vitamin C on fertility in cattle. He states that though ruminants *synthesise their Vitamin C requirements*, changes in concentration of Vitamin C in the blood plasma are observed during the mid to late heat in cows, showing that Vitamin C production follows the reproductive rhythm. It has been shown by some workers that Vitamin C injections have improved the reproductive capacity of the cow with regard to irregular heat, conception rate etc., however, it is still uncertain as to the exact role of Vitamin C in reproduction in the bovine.

### Vitamin D

#### CATTLE

Cows on Vitamin D deficiency feeds fail to show heat symptoms. Calcium-Phosphorus metabolism is under the control of Vit. D and as such deficiency of Vitamin D will show higher incidence of infertility. It has been shown in the continental countries that decreased fertility during winter months was associated with Vitamin D deficiency as a result of poor sunlight. Sun dried hay is rich in Vitamin D. If it is replaced by poor quality hay or green silage, it may cause Vitamin D deficiency particularly during winter months and cause disturbances in fertility. Under tropical conditions, usually the hay is exposed to intensive sunlight for long periods which naturally decrease the

quality of hay by lowering Vitamin A and carotene content to a considerable extent.

## Vitamin E

### CATTLE

The specific effects produced by Vitamin E also known as the "*Antisterility factor*" in reproduction in the bovines are not clear. There is close relationship between Vitamin C, E and cystine and their influence on fertility.

### SHEEP

Even when Vitamin E is considered essential in reproduction there is no documentary evidence except that ewes with Vitamin E deficiency will produce Vitamin E deficient lambs which may develop muscular dystrophy. The condition can be corrected by treating the lambs with synthetic tocopherol but not their dams (Thomson and Aitken, 1959). Baxter (1962) suggested that selenium contents may influence proper utilisation of tocopherol in the diet.

deficiency. Mahadevan (1962) mentioned delayed sexual maturity and irregular oestrus and oestrous cycles as a result of phosphorus deficiency. If phosphorus intake is low then a widening of Ca:P ratio can lead to still further depression of fertility. When Vitamin D status is high, the adverse effect of wide Ca:P ratio disappears. As against this, the feeding of excess phosphorus relative to calcium may render the endometrium particularly susceptible to genital infection.

The survey of farms carried out by Hignett and Hignett (1952) seems to indicate the possible adverse effects of sub-optimal levels of phosphorus or wide Ca:P ratios on fertility in absence of recognisable clinical symptoms of the deficiency. This is tentatively corroborated from the work of Ford (1956), Brochart (1956-1957) and Munro (1957).

## Copper

Elvehjem (1935) established the fact that copper is essential for haematopoiesis in a range of animals. The levels of copper in various tissues of the body are highly variable. The endocrine glands—pituitary, thyroid and thymus carry low levels of copper whereas liver, heart, kidney, hair and brain are examples of high copper concentration.

Sjollem (1933, 1938) in Holland showed that copper deficiency is a causal factor in a disease of sheep and cattle locally known as 'Lachsucht' characterised by diarrhoea, anorexia and anaemia. Bennetts and Chapman (1937) in Australia found that a demyelinating disease of lambs named, "Neonatal enzootic ataxia" was a manifestation of inadequate copper intake from the grazing and could be prevented by administration of copper to the ewes during pregnancy. Underwood (1962) mentioned that copper participates remarkably in a variety of functions in an animal body. It is vitally concerned in the process of pigmentation, keratinization of wool, bone formation, reproduction and myelination of the spinal cord in addition to that of haematopoiesis.

Newborn and very young animals except cattle and sheep are normally much richer in copper per unit of body weight than adults of the same species. Newborn levels are well maintained throughout the suckling period, followed by a steady fall. Copper concentration of the endocrine glands, the muscles, brain, heart and the skin are not generally affected by deficiency or an excess of dietary intakes. Whereas copper content of the liver, kidneys, spleen and lungs can be greatly increased by high copper intake and that of the liver, kidney, spleen, hair and blood are greatly

reduced in condition of copper deficiency. The liver is the main storage for copper in the body and level in the liver provides a useful index of the copper status in animal body. The liver copper levels are influenced by four factors viz. the species, age, nature of the diet and certain disease conditions. But sex does not seem to affect liver copper. Pryor (1961) observed that there is no significant change in liver copper changes during gestation. The liver copper status of the new born may be subnormal if the copper intake of mother is low as shown by Bennetts and Beck (1942). However, post natal copper supplementation usually rectifies the position. Olcraft and Uvarov (1959) have shown that dietary supplements of copper during pregnancy are effective in raising the copper levels of foetal liver to normal in conditions where copper deficiency existed in the mother. Underwood (1962) mentioned that in all species colostrum is significantly richer in copper than it is in the latter stage of lactation. This has been demonstrated by Elvehjem *et al* (1929) in cows and goats. Thomas (1937) in ewes observed that the addition of copper to feeds having adequate supplies have no effect on the copper content of milk. Similar mammary barrier is observed to exist with iron but not with zinc, manganese, iodine, nickel and cobalt whereas deficient intakes of copper are reflected in subnormal levels in milk of cows and ewes. Bennetts and Hall (1939) reported that low fertility in cattle has been associated with copper deficiency in several areas. Dutt and Mills (1960) reported that in rats copper deficiency results in reproductive failure with death and resorption of foetuses. Disturbances in oestrous cycle due to



copper deficiency have not been demonstrated except the one described by Sane *et al* (1958) who observed that low blood levels of copper in cows and heifers resulted in suppressions of oestrus and oestrous cycles

## CATTLE

Copper is vitally concerned in the maintenance of fertility. Copper deficiency in ruminants is not clearly understood. It is even known to occur in grazing animals on pastures where copper levels are in the normal range (5 p.p.m. DM). It may also be associated with high levels of Molybdenum.

Olcraft and Parkar (1949) and Underwood (1962) have shown that other factors also seem to be involved. Bennetts *et al* (1948) reported a high incidence of infertility due to copper deficiency. Anaemia and retarded growth were observed where copper levels of the pastures were less than 3 p.p.m. By giving copper supplements the condition was overcome. Mills (1967) has pointed out situations in which copper deficiency in young stock was severe and yet there were no adverse effects on the breeding performance. This is in contrast to observations made by Sane *et al* (1958) in their investigation for infertility in Gir herds due to copper deficiency. The deficiency completely suppressed the breeding performance with the consequence that in almost all heifers and cows ovarian function was suppressed with the result that ovaries were completely inactive and hard coupled with atonic condition of the uterus. The body colour changes were evidently marked in red coloured coats in varying intensity which changed to dirty brown and pale red (Achromotrichia). The

fertility was restored on supplementation of 10 gr copper sulphate and 2 gr of cobalt sulphate daily for a period varying from 6 weeks to 4 months. Mahadevan (1963, 64) reported the effect of 50 mg copper sulphate supplementation to the feed in 9 cows. The intercalving period in control group was 445 days whereas the period was reduced from 559 days to 348 days in experimental group the other conditions being equal. Kaikini *et al* (1969) observed beneficial effects of feeding copper-cobalt supplement in the daily feed of seven Sahiwal heifers (in the age groups of 1½ to 2½ years) in which ovaries were yet to start functional activity.

In a group of 40 pubertal anoestrous Gir heifers and 30 cows with postpartum anoestrous condition, feeding triple sulphate viz cobalt sulphate 2 mgm, copper sulphate 150 mgm and iron sulphate 1 gm daily for a period of 10 days and followed by a repeat course after 10 days induced ovulatory oestrus and pregnancy. The combination of trace elements appear to have produced beneficial effects in initiating ovarian activity by exerting synergistic action (Kodagali, 1978).

## SHEEP

Ewes suffering from copper deficiency may produce lambs showing ataxia or swayback condition. The condition can only be cured by supplementing the diet with copper during gestation. The deficiency may be simple due to low levels of copper or complicated with high levels of molybdenum or other unknown factors which interfere with absorption or utilization of copper. Wilson (1962) reported deficiency in ewes to occur on granular treated with heavy doses of lime.

### Cobalt

The occurrence of cobalt in animal tissues was demonstrated by Bertrand and Macheboeuf (1925). They detected minute amounts of cobalt in most of the organs of mammals and man. It was further demonstrated that cobalt is an essential nutrient and mice fed on supplemented diet lived longer. It has been proved by experiments that except in ruminants cobalt deficiency cannot be induced artificially. Cobalt is essential for the synthesis of Vitamin B<sub>12</sub> by the ruminal microflora and symptoms of cobalt deficiency can be overcome by administration of Vitamin B<sub>12</sub> alone. In certain cobalt deficient areas in many parts of the world, sheep and cattle become weak, emaciated anaemic and finally succumb. As against this, non ruminants like horses did survive on the same lands. In sheep, it was observed that the administration of 1 mg. of cobalt/day by mouth, resulted in a dramatic improvement in the appetite, body growth and haemoglobin levels of coastal sheep. Better results have been observed with cobalt in combination with copper by Marston *et al* (1948). In sheep and cattle, it is observed that due to cobalt deficiency, there is emaciation, listlessness, blanched mucosa and pale and fragile skin. The body of severely affected animal is a picture of extreme emaciation, often with a total absence of body fat. Lee (1949) reported that grasses usually have low concentration of cobalt than legumes or herbs grown under the same conditions.

Normal pastures and fodders vary to a great deal in cobalt content but lie within a range of 0.1 to 0.3 p.p.m. Mapson (1933) observed that cobalt content of colostrum is almost 10 times higher than in milk. The average level being about 5 mg/L.

### Molybdenum

Molybdenum in low concentration is present in plant and animal tissues. Bortels (1930) has shown that molybdenum is an essential nutrient for growth of azotobacter, a nitrogen fixing soil bacteria. Anderson (1956) described that molybdenum deficient soils have been discovered in many parts of the world and striking crop responses were demonstrated with molybdenum supplementation. Ferguson *et al* (1938, 1940 and 1943) reported that disease known as 'teart' — amongst grazing cattle has been recorded in England with drastic diarrhoea as a typical symptom. This is said to be due to ingestion of excessive amounts of molybdenum from the herbage grazed. Brouwer *et al* (1938) reported a similar condition in Holland amongst cattle grazing in copper deficient areas. Treatment with copper gave favourable results. Dick and Bull (1945) successfully treated cases of chronic copper poisoning in sheep in Australia by use of molybdenum. The above work tends to indicate the profound influence of molybdenum on copper metabolism. Unlike iron and copper, molybdenum does not appear to have a tendency to accumulate on high levels of intake. Most of it is excreted through urine.

Thomas and Moss (1951) have shown that high intake of molybdenum causes disturbance of phosphorus metabolism in some animals such as lameness, joint abnormalities, osteoporosis etc. It has also been reported that cows subjected to such conditions fail to conceive and young bulls exhibit a complete loss of libido. There was marked damage to both the interstitial cells and the germinal epithelium. Spermatogenesis was also suppressed.

Sheep are less affected by molybdenum toxicity than cattle. Horses and pigs are most tolerant. In all species retardation of growth and loss of body weight invariably is the result of high molybdenum intake. Inghall *et al* (1915) have reported that diarrhoea is an important feature of molybdenosis in cattle. Anaemia and loss of coat colour follow prolonged intakes.

It has been proved that a quantitative relationship exists between copper and molybdenum. High levels of molybdenum which lead to scouring in cattle in spite of adequate levels of copper can be controlled by massive oral doses of copper to counteract the ill effects of high molybdenum intakes. Dick (1954) has shown that there is an influence of molybdenum in copper metabolism. The copper status in animal body can increase or decrease depending on whether the intake of molybdenum is low or high. High levels of molybdenum feed contents decrease the copper concentration in the liver only to a certain level and beyond this the liver copper levels do not drop but the physical symptoms of copper deficiency become apparent. Other dietary factors like protein, manganese and sulphates are also known to influence the interrelationship between molybdenum and copper. Westerfield and Richert (1953) have found that legumes, cereal grains and some green leafy vegetables are good source of molybdenum. Beck (1962) reported that the grasses usually have higher concentration of molybdenum than clover growing with them whereas reverse is the condition with most of the other trace elements.

### Manganese

All tissues of the animal body contain manganese in very low concentration. It

has been found to be an activator for some enzyme systems. Sato and Murita (1932) reported that cow colostrum is several times richer in manganese than normal milk. The normal level of manganese can be increased substantially by feeding additional quantity of manganese to the cow. As stated by Underwood (1956) under certain dietary conditions the egg contains insufficient manganese to permit normal hatchability and subsequent development of chick embryo. Schaible *et al* (1938) observed that manganese deficiency in cattle, sheep, goats or horses under natural grazing or stalled conditions is extremely rare. Feeding greens is said to have an added advantage of raising the manganese levels. Grashuis *et al* (1953) reported that the main symptoms of manganese deficiency are poor body growth, leg deformities with knuckling, poor fertility, frequent abortions and dead, dry and brownish hair.

### CATTLE

Hignett (1956), Bentley and Phillips (1951), Munro (1957), Dyer (1965), Rojas *et al* (1965), Wilson (1965) have described the influence of manganese on fertility and have reported better conception rate by manganese supplement. Bentley and Phillips (1951) reported that the ovary particularly appears sensitive to low levels of manganese and the largest decrease in manganese content is observed in this tissue. Dyer (1965) and Rojas *et al* (1965) reported birth of deformed calves due to manganese deficiency; joints were found enlarged and legs twisted. Thompson (1957) stated from experimental evidence that a level of about 25 ppm of manganese in the diet meets with reproductive requirements and as the manganese contents of pasture is usually in the

region of 50 to 100 p.p.m. there are remote possibilities of occurrence of manganese deficiency in animals grazing on pastures. Wilson (1966) reported improved fertility from supplementary manganese in case of pasture which range from 50 to 75 p.p.m. It is quite likely that in some type of herbage, manganese levels may be poor particularly in the ones from heavily limed pastures.

#### SHEEP

No information is available on the role of manganese in the reproduction in sheep. Underwood (1962) reported that a deficiency of manganese may affect reproduction in laboratory animals, pigs, and cows.

#### SWINE

Johnson (1943) observed that diets deficient in manganese content may not affect the growth but reproduction in such cases is usually poor. Inferior development of the udder is also seen. Newland and Davis (1961) found no significant adverse effects on the reproductive capacity of gilts fed on low levels of manganese 6 p.p.m. Plumlee *et al* (1956) observed irregular oestrous cycles in gilts fed from weaning and throughout growth on feeds containing only 0.5 p.p.m. of manganese. Resorption of foetuses occurred and piglets born were small and weak. It is thus clear that though a deficiency of manganese has severe effects on reproduction, the actual concentration required for optimum levels of reproduction in the swine are very small, about 5 p.p.m. or even less.

#### Zinc

It has been shown by Birkner (1919) that Zinc is essential in the normal growth processes of higher animals. He

reported that the milk and yellow of the eggs contain high quantities of Zinc. It has been shown by Keilin and Mann (1939) that the presence of zinc is highly essential for certain enzymatic activities. Soils deficient in zinc show poor growth of the pasture. In the cat and pig there is no fall in the whole body concentration of zinc during suckling. In the rat and the pig there is a substantial rise from the newborn levels during the suckling and the early stages of growth (Spray and Widdowson, 1951). This seems to indicate that young mammals get their zinc requirements from colostrum and milk. Colostrum is four to five times richer in zinc than milk. Zinc is present in all living cells. Hair pigmented tissues of the eyes and the bones carry high concentration of zinc than other tissues and organs of the body. Zinc has a tendency to accumulate in the bones. Bertrand and Vladesco (1921) have shown that there is a high concentration of zinc in testis of man and ram. However no differences were observed in the zinc content of ovaries during the period of sexual activity. Davis *et al* (1952) observed that, there is increased absorption of zinc in pregnant cows and rats during the last third of pregnancy. Colmano and Fiori (1951) observed that deficiency of zinc may lead to male infertility and to the reduction of size of testis. Zinc is concerned in some way or the other in the testicular development and its function. Plants normally contain fairly high content of zinc, as such its deficiency has not been observed in farm animals and grazing stock. Even in zinc deficient soils, there is a reduction in bulk of the herbage produced but not in the zinc content.

It is evident from the observations made by Pond and Jones (1964) that zinc intake does affect reproduction in gilts.

The concentration of zinc in feeds has to be below 35 ppm even in the presence of high level of calcium—a factor which has a tendency to reduce availability of Zinc.

### Iodine

Baumann (1896) observed that iodine is a normal constituent of the body tissues. Von Fellenberg (1926) has apparently shown that food and not the water is the main source for iodine both in man and animals. It is said to be unique among the trace elements since it functions solely as an indispensable constituent of thyroxine. Underwood (1956) mentioned that the concentration of total iodine in the thyroid gland varies widely with the intake of iodine with glandular activity, age and individual variations. However, there is no evidence to show that it varies significantly with sex in any species. The concentration of iodine in the thyroid glands is closely related to its functional capacity.

It has amply been proved that iodine has a marked effect on the rate of growth, reproductive performance and lactation in farm animals. Adequate dietary daily intake is therefore of considerable importance. Carroll *et al* (1951) have shown that hypothyroidism is associated with dwarfism and hence low rate of gain in weight. Iodine exerts its action only through thyroid and is concerned with metabolic processes and controls

turation, especially of the skeletal tissues, the reproductive organs and the appendages of the integument.

There is close relationship between the functioning of the pituitary and the thyroid as thyrotropin stimulates the thyroid. Reineke and Turner (1944) and Roche *et al* (1955) have mentioned that in the cow and the goat the thyroid of the foetus may contribute to the thyroid requirements of a thyroid deficient mother.

Thyroid gonadal interrelationship is markedly observed in some types of birds. In Brown Leghorn males, thyroidectomy is followed by a long period in which testes remain small with no spermiogenesis. The comb decreases in size, moulting is inhibited and the characteristic male plumage is lost. Oestrogen administration alone to such birds does not induce the female plumage pattern as is observed in normal males. This proves that there is a synergistic action of the thyroid and ovarian hormones (Bilvaiss, 1947). There is a reduced egg production in the hen due to thyroidectomy. The seasonal cycle of egg production in poultry is related in part to seasonal variations in thyroid activity. However Wolf *et al* (1946) and Turner *et al* (1945) mentioned that poultry can withstand iodine deficiency to a considerable degree without any marked effect on egg production and hatchability.

Hignett (1952), Olcraft *et al* (1954) and Moberg (1959) observed that due to iodine deficiency, foetal development may be arrested at any stage leading to early death and resorption, abortion, still birth or birth of weak young ones. With prolonged gestation and parturition, retention of foetal membranes is also observed. Maqsood (1952) reported that the thyroid gland has an important part in the maintenance of male fertility. Hignett (1952) and Jovanovic *et al* (1953) also reported that there was decline in libido and also deterioration in semen quality due to iodine deficiency in bulls and stallions.

#### CATTLE

Thyroid function is vitally concerned with production. Failure of oestrous in thyroidectomised cows suggests a close relationship of thyroid with the ovarian function (Spielman *et al*, 1945). In iodine deficient areas, favourable results have been reported in repeat breeding cows by feeding 20 to 40 gr. of an organic iodine preparation daily from 8 to 18 days before the onset of oestrous (McDonald *et al*, 1961). Hignett (1952) and Jovanovic *et al* (1953) reported irregular or suppressed oestrous in cattle causing infertility and sterility due to iodine deficiency. Heifers fed on large proportion of Kale resulted in anoestrous condition with small inactive ovaries (David, 1956). Mahadevan (1962) has enumerated the different effects of iodine deficiency on the reproductive tract including a high incidence of post-partum genital infections. Iodine deficiency has been recorded in certain parts of Himalayan regions. Effect on fertility however, depending on variations in iodine levels, is not clearly known.

#### SHEEP

Falconer (1963, 1965) observed that thyroidectomised ewes exhibited normal oestrous cycles and conception rates, but the lambs born had reduced pre and postnatal viability. Setchel *et al* (1960) reported reduced viability in lambs as a consistent feature while hairless condition was seen occasionally. Ewes entirely on Kale feeding are reported to produce a small lamb crop as compared to those partly on grass and partly on Kale and also with ones receiving iodine supplement, alongwith Kale (Shand, 1952). Sinclair and Andrews (1959) observed neonatal mortality in lambs from ewes fed on goitrogenic Kale. Such ewes respond favourably to iodine administration during pregnancy. Sinclair and Andrews (1954, 1959) and Andrews and Sinclair (1962) observed that lambs which died from the Kale fed group had enlargement of thyroid and low thyroid iodine content. David (1965) found that oestral activity was not affected by Kale feeding, even when it was continued over 10 weeks. Ovulation and fertility appeared satisfactory, though Kale is goitrogenic. However there was indication of increased embryonic death after implantation with consequent increase of services per conception. There was marked adrenal enlargement in the hypothyroid animal which may be associated with the reproductive failure. Williams *et al* (1965) observed embryonic mortality in Kale fed ewes slaughtered from 18 to 22 days after the service. Care (1954) reported linseed meal as a source of goitrogen. Ewes fed 20% linseed meal in their ration during gestation produced a fairly high percentage of goitrous lambs. Supplementation of iodine proved beneficial to overcome the effect.

It is reported by Maqsood (1952) that prolonged administration of thiouracil to ram lambs prevented the onset of sexual maturity. Brooks *et al* (1961) observed that the mature thyroidectomised rams maintain their libido and fertility. Warwick *et al* (1948) observed that rams fed on excess thyroproteins were found to give poor quality of semen. Bogart and Mayer (1916) reported a seasonal decline in semen quality in rams associated with a mild hypothyroid state. It may thus be seen that a disturbance of thyroid activity in the ram leads to disturbances of the reproductive process.

### Selenium

It appears from the literature that selenium plays an important role in reproduction in the sheep. By feeding selenium 1 to 25 mg it has been observed by Hartley (1963) that it reduces the percentage of barren ewes. Kuttler and Marble (1960) reported that by supplementing selenium to ewes during gestation beneficial results have been observed in preventing muscular dystrophy (white muscle disease) in lambs. Blaxter (1962) has pointed out that though naturally occurring Vitamin E deficiency appears to respond to Vitamin E supplement primarily it is responsible to shortage of selenium. The exact functional association of Vitamin E with selenium is not still clear. Muth *et al* (1958), Proctor *et al* (1958), McLean *et al* (1959) and Hartley and Grant (1961) have shown that trace element selenium is largely responsible for growth and reproduction particularly in ruminants.

It is reported that in New Zealand in some areas striking growth responses to selenium have been observed. In experimental trials in selenium deficiency in New Zealand, the mortality rate of

lambs was reduced from 27% to 8% by supplementation with 5 mg of selenite in three doses at commencement 2 weeks and 6 weeks interval. This treatment also led to highly significant increases in the live weight gains. In some areas in New Zealand experiencing white muscle disease there is an associated problem of infertility. With selenium deficiency the normal pattern of oestrus and oestrous cycle is not altered in majority of cases and ewes do conceive normally but later it appears that the lambing rates were reduced considerably. Factors responsible for such a condition are not clearly known. It appears that selenium deficiency may result in transient form of infertility which can be corrected by administration of selenium in proper doses.

The minimum dietary levels of selenium conducive to optimum growth, health and reproduction is not yet clearly known because it is required in very minute quantities and its interaction with other components of diet are not understood. The total selenium levels in herbage may reflect the levels available to the ruminants because the trace mineral is present in different chemical forms and utility of each is not properly understood.

### Selenosis

Selenium toxicity is dependant on the level of selenium instead of the duration of intake and the nature of the rest of the feeds. Alkali disease is the chronic form and blind staggers is the acute form.

In horses and cattle the first noticeable symptoms of selenosis is loss of long hair followed by soreness of the feet. In affected areas foals and calves are at times born with deformed hooves or may

develop these during suckling. Pigs fed with toxic grains show lameness, irregular growth of the hooves and emaciation due to poor intake of food, slow growth and loss of body hair. Anaemia is common in all species. In poultry there is fall in the egg production. The eggs show poor hatchability. Franke and Tully (1935) reported that in Poultry, selenosis has resulted in deformed embryos characterised by missing eyes and upper beaks, distorted wings and feet and a peculiar "wiry" condition of the down. Thus though the eggs are fertile, the hatchability is low in spite of low concentration of selenium so as to cause obvious symptoms in other classes of livestock. Rosenfeld and Beath (1954) reported that in rats, selenium can interfere with normal development of the embryo. Similar condition has been reported by Wahlstrom *et al* (1955) in pigs, and Rosenfeld and Beath (1947) in sheep and poultry. Rosenfeld and Beath (1954) observed that the effect of selenium on the reproductive performance is highly marked in the female than in the male. Wahlstrom *et al* (1955) have mentioned that in young sows 10 p.p.m. sodium selenite, decreased the conception rate, increased the number of services per conception and increased the proportion of piglets dead, small or weak at birth. For diagnostic purposes the levels of selenium either in blood or hair have been found useful.

### Fluorine

The actual impact of fluorine on fertility is not very clear. When compared to other trace elements there is a narrow margin between optimum levels of intake necessary for physiological requirements and the one which might induce toxic symptoms, even with slight increase.

It is not clearly known as to whether it is essential for the physiological requirements of various types, except for the dental health. However, fluorine toxicity is widely known. Even low intake of fluorine has no appreciable effect on fertility. Harvey (1952) reported that at very high fluorine intake the placental transfer of fluorine is sufficient to affect the newborn. Van Rensberg and De Vos (1966) from their studies on Africander cattle concluded that for optimum levels of fertility, the fluorine intake should be less than 5 p.p.m. Decline in fertility was observed at higher levels.

### Iron

Iron is an important constituent of haemoglobin which contains the bulk of the body iron. Iron content of the animal body varies with the species, age, sex, nutrition and health status. The species differences in the total body iron concentration appears to be small in adults but appreciable differences are observed in respect of new born (Widdowson, 1950; Granick, 1951). Spray and Widdowson (1951) observed that there is relatively little iron in the body of pigs at birth as compared to that in rabbits. This is also related to the lower content of iron in the liver. But in contrast, the newborn rabbit has a much higher content of iron in the body due to its large iron stores in the liver. Otis and Smith (1940) have shown that female rats have a higher total body iron than the males.

Milk is an extremely poor source of iron and administration of iron supplement to cows or sows are not capable of raising the iron content of their milk beyond the levels above normal. This is contrast to the observations on zinc, manganese, cobalt and iodine. Under-



wood (1956) mentions that cow's colostrum contains 3 to 5 times the iron of normal milk.

Iron deficiency has not been encountered in farm animals except pigs in which iron deficiency leads to very high mortality on account of piglet anaemia.

### MISCELLANEOUS TRACE ELEMENTS

#### Aluminium

It is not known if there is evidence to the effect that aluminium has any essential function in plants, animals or microorganisms.

#### Arsenic

Arsenic has a small but significant influence on the growth in chicks, turkey poults and piglets. Arsenic does not readily pass the mammary barrier.

#### Strontium

Radiostrontium is well absorbed by the plants as compared to animals. It is accumulated in bones and transmitted to foetus by the mother. Strontium is a regular constituent of soil, plants and animals. It has been reported by Schröter and Schropp (1937) to be a plant growth stimulant.

#### Barium and Strontium

Rygh (1949, 1951) has mentioned that the omission of either barium and strontium from the mineral food caused depression in growth of rats and guinea pigs while strontium promoted mineralization of bones and teeth. Barium has the effect of decalcification of osseous tissues. Though both are reported to stimulate plant growth, none has been found to be essential to growth and development of plants.

#### Boron

It occurs widely in plant tissues. The role of boron in animal nutrition is not clear.

#### Bromine

Marine plants are richer in bromine as compared to land plants. This is similar to iodine. Cereal grains are the lowest in bromines of various plant materials. Bromine content of cow milk is very variable. All animal tissues other than the thyroid glands are usually 50 to 100 times richer in bromine as compared to iodine.

#### Cadmium

Cadmium is present in plant and animal tissues mostly in the order of 1 ppm or less (Klein and Wichman, 1945). The cortex of the horse kidney contains high levels of cadmium.

#### Chromium

All plant and animal tissues contain chromium in variable quantities.

#### Nickel

All plant and animal tissues contain nickel. This element is present in the soil and plants in a much higher proportion than in animal tissues. Palmer and Underwood (1936) observed that nickel could partially replace cobalt in the treatment of cobalt deficiency of sheep. Ellerman and Perking (1935) and Sugai (1944) have shown that though nickel is known to activate a number of enzymes *in vitro*, its functional significance in living animals is yet to be confirmed. Walker and Vallee (1959) mentioned that along with a number of metals, nickel is consistently present in RNA. Feeds of plant origin are richer in nickel compared to those of animal origin.

### Rubidium

Rubidium affects growth and reproductive performance if present in toxic levels. All the soft tissues of the body contain relatively higher concentration of rubidium without accumulation in any particular organs or tissues. Soyabean is the richest source.

### Silicon

It is found so abundantly in Nature, in soil and plants that good intake is automatically assured. Species variation in silica content are considerable. Archibald and Fenner (1957) reported that substantial quantities of silica are found in cows milk but they are little influenced by dietary silica intakes.

### Titanium

In almost all species, titanium is always present in living tissues but in low concentration in all cells.

The exact role played by the miscellaneous trace elements viz. aluminium, arsenic, barium, boron, bromine, cadmium, chromium, nickel, rubidium, silicon, strontium, titanium and vanadium on fertility is not clearly known, nor it is clear if their toxicity leads to subfertility or infertility.

### Oestrogens

Certain plants, grasses and pasture herbage contain oestrogen like substances which are reported to influence the function of reproduction. Bennetts (1946) and Underwood and Shier (1951) reported a breeding problem in ewes, that was encountered in Australian grazing pastures which had the subterranean clover as the most predominant species. Dwalganup strain of this clover caused infertility, dystokia, relaxation of the

pelvic ligaments and prolapse of uterus. Survey reports in Western Australia revealed that 70% of ewes failed to conceive and also there was further influence on lambing percentage which reduced by 30 to 40%. Moreover about 15 to 20% ewes died during parturition. In majority of ewes, there was cystic glandular hyperplasia of uterus associated with this condition. Mammary development and lactation in virgin ewes and wethers were the other changes noticed. The overall effects appear to cause, permanent physiological changes which were of an irreversible nature. This was evident from the affected ewes though transferred to non-clover grazing, did not return to fertility but continued to show infertility, dystokia and changes leading to cystic hyperplasia of uterus. Bradbury and White (1951) proved that oestrogens or oestrogen-like substances are present in subterranean clover by isolation of 'genistein' from it. Curnow and Rossiter (1955) have summarised that the presence of 'Genistein' or Gemstein like substances are found in all strains of subterranean clover and however several other clovers such as the red and white clover, are reported to contain very little of such type of compounds.

### Nitrates

Garner *et al* (1958) observed decreased viability in piglets farrowed by sows fed on high level of nitrates.

It is evident from the investigations of Teague (1955) that gilts fed on lucerne expressed fairly good rate of ovulation as compared to controls fed on concentrate mixture containing all the essential nutrients including Vitamin A and D. Feeding of Lucerne therefore has great influence on normal fertility.

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# Chapter 52

## Sexual Behaviour in Domesticated Animals

### I. SEX DRIVE

#### 1. The Male

Sex drive in the male animal is frequently referred to as libido. The drive matures at puberty and persists, in fairly constant degrees throughout the remainder of the animal's life. Libido is dependent on the production of testosterone but in the individual its optimum degree of manifestation is determined by, inherited characteristics. Libido varies slightly between different age groups. The sex drive may alter in consequence of physical changes and also with acquired inhibitions associated with adverse experiential factors. Under natural conditions poor libido is self-limiting—imparting little of its inherent basis to the next generation—but domestication can allow its propagation. Nutrition can apparently exert some slight influence on libido. High planes of nutrition can inhibit testosterone secretion in some young males, while gross underfeeding can also impair libido. The genetic basis of libido is evidently great according to very strong circumstantial and experimental evidence from many quarters. Libido is therefore largely inherent and genetic in nature.

Whilst the strength of sex drive affects fertility in the male, it is important to emphasize that there is no significant correlation between sexual behaviour and semen quality. In any assessment of the breeding ability of a sire, an adequate examination must therefore note the efficiency of sexual behaviour, in addition to semen characteristics.

Libido manifests itself in a variety of behavioural components which comprise male courtship. A behavioural component of libido common to most ungulates is the "olfactory reflex" or flehmen. In this, the animal fully extends the head and neck, contracts the nostrils, and raises and curls the upper lip. It occurs most usually subsequent to smelling urine and nosing the female perineum and is almost certainly a form of odour testing.

"Prompting" and "tending" are often shown by the male while consorting with the female before and during oestrus in the latter. Firm standing by the female is the positive response to "nudging" by the male subject, and provides reciprocal stimulation. Nudging can be seen in some form, in the pre-coital behaviour of most ungulates. It is seen in the stallion for example which tests oestrus in the mare

not only by smelling her, but also by biting and nipping her over areas of the body, working from the hind-quarters towards the neck. Pre-coital nudging is also commonly observed in the male goat.

Once mating has occurred, freely associating partners often exhibit the so-called "tending bond". Both sexes contribute to this temporary alliance thereby facilitating repeated mating and ensuring optimum conditions for fertilization.

## 2. The Female

The view is now taken by many that the term oestrus should be reserved to describe behaviour and not physiology although the normal mating state of the female obviously has both behavioural and physiological elements. The recognition and detection of ovarian changes in oestrus has made possible the discovery that, in many cases, the ovaries can undergo oestrous changes without the subject showing signs of oestrous behaviour. This further encourages separate recognition of the two aspects of the female mating state.

Behaviour in general is altered when the mating drive in the female subject is evoked. The usual routines of behaviour are disturbed during overt oestrus and typically there is a reduction in ingestive and resting behaviour, while locomotor, investigative and vocal behaviour are increased. All of this is secondary to the essential character of oestrus, namely, receptivity to mating.

The mounting behaviour between females so typical of oestrus in the bovine subject is seldom observed in mares. The oestrous period in the

horse is relatively long, lasting about six days on the average. During all this time a variety of symptoms of oestrus is shown. As with other species, the intensity of the mating drive varies a good deal from one instance to another. The mare in heat is seen to assume a staling stance very frequently (normally a mare will urinate only three or four times in a twenty-four hour period). During this frequent straddling, urine is spilled in small amounts; the clitoris is exposed by repeated rhythmic elevations. The company of other horses is sought, and particular interest is shown towards the male. In the presence of the stallion, the mare in heat will orientate her hindquarters towards it and adopt a stationary stance. It is found, however, that some mares of fractious temperament, though in heat, may kick forcefully on being mounted by the stallion. Variations in the intensity of the oestrous drive has also been well noted in cattle. Some oestrous sows show the "rigid stance" readily, others will only show an oestrous display when given boar stimulation.

## 3. Biostimulation and Sex Drive

It is now realised that optimum oestrous response in the behaviour of several animals (e.g. the mare and sow) is elicited after male prompting. Various aspects of such "biostimulation" have come to light only recently in the "Whitten" type of effect (Whitten, 1956). The true Whitten Effect is a phenomenon in which synchronisation and induction of oestrus in mice can be achieved by the introduction of a male mouse into a colony of female mice. This might appear to have little relevance in considering large animal reproduction but there is increasing evidence that this type of phenomenon may be

manifest in a variety of mammalian species

Oestrus can be prompted or induced in a variety of ungulate species by genital stimulation in ways which resemble phenomena of the Whitten type. Much of the evidence of oestrus induction by genital stimulation has come to light only recently. For example it has been reported that local stimulation of the genitalia can have a definite effect on the induction of oestrus in horses and cattle. In the mare local genital stimuli can apparently have some effect on inducing overt oestrus. One Russian experimenter Prahov (1959) by the use of five methods of genital stimulation e.g. stimulation of clitoris or vagina induced oestrus in eighteen out of thirty two anoestrous mares. Behavioural forms of genital stimulation are evidenced by nuzzling, nudging and licking about the perineal region in pre-coital behaviour. It would seem therefore that the induction of oestrus in a variety of animals is due not only to endogenous and central stimulation but also to exogenous and peripheral stimulation by odour, sound, sight and touch. The complete natural stimulus is evidently of a complex nature with olfactory, ocular, auditory and tactile stimuli being supplied by association with an active male of the same species. This concept represents something of a progressive departure from the formerly held view when oestrus in the large animals was commonly considered to be essentially under internal control.

#### 4 Timing of Oestral Drive

The mare is the best known example of a species showing early oestrus after parturition. This early heat is called the foal heat and occurs on the ave-

rage about nine days after the birth of the foal. The foal heat is often short but is commonly displayed intensively. Some considerable variation occurs in the occurrence of foal heats. The earliest heat noted in a Polish study was on the fourth day and the latest on the one hundred and thirty second day after foaling. It was observed that 65%—69% of mares showed oestrus before the twentieth day after parturition and 70—80% of all mares studied came into heat by the thirtieth day. Cattle normally show oestrus two months after calving. Sheep and goats show oestrus seasonally.

No other large animal has a normal oestrous cycle so notoriously irregular in duration as the horse. This irregularity is found in the horse at all latitudes. It can be taken as a fixed characteristic of the species. A common range in the duration of oestrus in the mare is four to ten days. Some observers such as Hammond (1960) have noted that shorter heats more often occur as the breeding season progresses. Oestrus in the bitch recurs after 6-9 months intervals irrespective of the time of year.

The two common natural conditions responsible for anoestrus are seasonality and pregnancy. Echstein and Zuckerman (1956) report that heat may occur occasionally during gestation in some animals. The author has had experience of mares showing heat while in advanced pregnancy. As to the incidence of such events it has been estimated that as many as 2% of pregnant cows show oestrus during pregnancy. Jolani (1960) found that 3—5% of water buffalo does showed behavioural oestrus while pregnant. Oestrus in pregnancy is not uncommon in donkeys.



## 5. Combining Sex Drives

Mating is the consequence of the sex drives combining. The impetus of mating is jointly the emergence of oestral drive in the female and the activation of male drive. The timing of mating is so arranged that spermatozoa are introduced into the female genital tract before the ovum is liberated from the ovary—a requirement of fertility. Copulations are therefore centred mainly in the earlier part of oestrus, with some fewer repetitions of mating occurring in the later part. The frequency of repetition varies enormously, differences in frequency being much greater between individuals than between species and clearly dependent on sex drive intensities. As a general rule, the rate of repetition diminishes in the middle period of the day and in late oestrus. These drops in sex drive help to conserve energy and sperm reserves in the male. Mating is not, therefore, a totally committed force in species survival, although it is the basis of it.

Patterns of mating behaviour of increasing complexity have evolved in mammals; these patterns are essentially concerned with stabilising the mating relationship. The enactment of mating patterns depends on the strength of sex drive plus the strength of the stimulus from the opposite subject. Mating activities are basically instinctive, but are also partially learned. The view has been expressed that the need for learning in mating behaviour is apparently greater in the male than in the female, but it is now recognised that heifers and maiden sheep, for example, are not always efficient in converting their oestrous drives into proper oestrous behaviour.

## II. MANIFESTATIONS OF SEXUAL BEHAVIOUR

### 1. Male

Many precoital components of behaviour seen, tend to be species specific. Sheep and goats, however, have items of courtship behaviour in common. These include: nosing of the female's perineum, nudging the female, flehmen, flicking out of the tongue, striking out with a forelimb and low-pitched bleating sounds. In addition to these behavioural features, the male goat also spills small quantities of urine particularly on to his forelegs. Buttings of the females hindquarters is also occasionally seen in both of these species. False mounting attempts are sometimes shown by rams and billy goats; this behavioural feature is also seen in horses. Bulls often pump their tailheads up and down during pre-coitus and during the same period may pass small quantities of faeces.

There are, however, certain major behavioural activities common to all male farm animals. These are: threat displays, challenges, territorial activities, female seeking and driving and female tending. These behavioural activities tend to flow into each other.

- (a) Threat displays are usually produced by animals in a static posture. The threat display of the bull is in fact a physiological state of fight or flight. In this state the animal arches his neck, shows protrusion of the eyeballs and erection of hair along the back. During the threat display the bull turns his shoulder to the threatened subject. A buffalo bull becomes very ferocious at the sight of other buffalo bull and fight invariably ensues.

- (e) "Nudging" in some form or other can be seen in the precoital behaviour of all the farm animals and is prominent in courtship behaviour (Fig. 169 a, b). Nudging behaviour prompts the female to move forwards. In oestrus the female responds to this stimulation by adopting a stationary stance, so facilitating mating. Firm standing is the positive response to nudging and provides reciprocal stimulation for the male. Rams nudge by pushing with their shoulders and also striking the hindlimbs of the ewes with their forefeet. Butting is another form of nudging shown by all the ruminants including bulls, rams and goats. In buffalo bulls, butting is more pronounced than in other ruminants. Boars "root" sows.
- (f) "Tending" behaviour is displayed by the farm animals when opportunities permit. The male maintains close bodily contact and association with the female whilst grazing near her. Both sexes contribute to this temporary alliance. In the tending-bonds of most of the farm animals there are phases when the male animal rests his chin over the hindquarters of the female. This "chinning" behaviour is best seen in cattle and buffaloes but it also occurs in other species (Fig. 169c).

## 2. Female

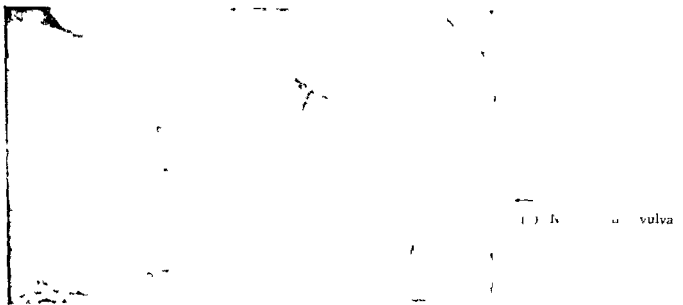
The mating state of the female animal is termed oestrus. Although the term oestrus applies principally to behaviour it must be acknowledged that it also describes some internal physiological processes. Although the two facets

of oestrus can occur separately this is rare and it is normal for them to exist simultaneously. When oestrus is shown behaviour in general changes and many of the animal's usual routines become disturbed and there is often an alteration and reduction in feeding and resting patterns. These are secondary to the essential characteristic of oestrous behaviour which is acceptance of the male. Variations in the manifestations of oestrous behaviour occur from one species to another.

The behavioural signs of oestrus in cattle include the following:

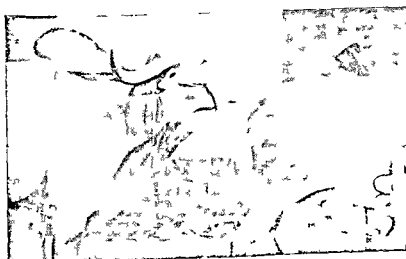
- (a) There may be an increase in what could generally be termed as excitement.
- (b) The oestrous cow bellows increasingly.
- (c) Grooming activities, in the form of licking other animals, are also increased.
- (d) Typically the oestrous cow indulges in a great deal of mounting of other cattle. When several cattle in a group have been prompted to mount each other, through the initial activity of the oestrous cow, it may become difficult for an observer to identify the oestrous cow in the group.
- (e) When one animal in particular is standing to be mounted by others that is the animal in oestrus.
- (f) Oestrus lasts for a period of 12 to 24 hours and it is commonly observed to be of shortest duration in younger cattle.

Oestrous behaviour in mares shows a range of characteristics peculiar to this species. The intensity of the oestrous drive varies probably more than in any

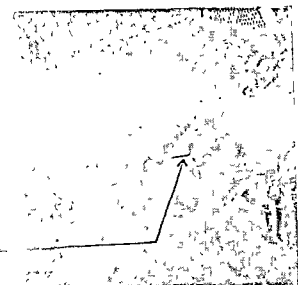


( ) vulva

(b) Licking the vulva



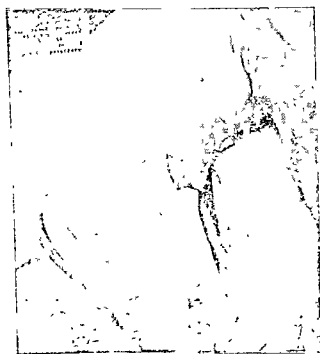
(c) Sniffing and chinring  
(Flehmen)



mounting and service. Partial and  
progressive intromission. Tail raised.



(f) Slight slackening of the grip of  
fore limbs. Partial withdrawal of penis.



(e) Vigorous thrust. Forceful muscular  
contractions. Straightening of hock  
joint.



(g) Grip of fore limbs slackened. Back  
joint lost its rigid arching. Hock  
becomes angular. Progressive with-  
drawal of penis

of the other farm species. A mare in oestrus typically adopts a maiming stance. During these periods of straddling mucoid urine is ejected in small quantities. Following this the animal maintains the straddling stance with the hindlimbs abducted and extended. The tail is elevated and arched up from the perineum. The heels of one or other hind hoof are commonly seen to be tilted up off the ground so that only the toe of that hoof remains touching the ground. Whilst this stance is maintained the animal shows flashing of the clitoris by repeated rhythmic eversion of the vulva. The duration of equine oestrus is 1 to 6 days on average but varies a great deal lasting only 1 day in some and up to 20 days in others.

The ewe shows discrete behavioural evidence of oestrus. Heat in this animal is extremely difficult to detect if there is no ram with the ewe. When a ram is present the ewe coming into oestrus will usually seek out his company and consort with him for a period of many hours before true oestrus commences. Ewes in oestrus frequently initiate the first sexual contact with rams and thereafter follow the rams about in their grazing movements as long as heat persists. Although the normal period is recognized as being just over 24 hours oestrus can last for up to 3 days in some ewes. Mutual riding among ewes, one of which is in oestrus, has not been reported.

In goats the signs of oestrus are very marked indeed. The female in oestrus shows rapid tail wagging actions during which the upright tail quivers very rapidly from side to side. This tail action resembles flagging and is frequently repeated throughout oestrus. Oestrus lasts on average just over 24 hours. During this period the female goat eats less

than usual, has a tendency to roam and bleats very frequently and loudly.

In sows oestrus is shown by ear pricking in short eared breeds and by the characteristic stationary stance which is adopted readily when pressure is firmly applied to the sow's back. Mutual riding takes place among sows when one or more is in oestrus but this is of less common occurrence than in cattle. Oestrus lasts about 48 hours.

Oestrus in the bitch is heralded by a long pro-oestrous phase. In oestrus proper the bitch responds to the approach of a male dog by taking a passive stance and arching the tail to one side. Oestrus lasts about 5-7 days and repeated mating will be permitted by most bitches throughout this period. Some bitches display an oestrus response only in respect of certain dogs. This idiosyncrasy of oestrus is a canine peculiarity which causes breeding difficulties.

Cats in oestrus vocalize in a characteristic fashion (caterwauling?). They also roll and tread with their hind feet while the tail is elevated and quivering. Concavity of the spinal column (lordosis) is the most pronounced feature. These behavioural signs of oestrus are occasionally confused with colic. Oestrus terminates soon after mating has occurred otherwise it may continue for a variable period, about ten days commonly.

#### ONSET AND FAILURE OF OESTRUS

The onset of oestrus in cattle may occur at any hour of the day or night but more often the latter so that there is a higher frequency of oestrus during the night. Similar diurnal variation has been reported in sheep. It has been noted that approximately 70% of ewes commence oestrus during the night.

Anoestrus is the condition in which the female animals fails to show cyclical recurrence of oestrus. Anoestrus normally occurs, of course, when the animal is pregnant or when it is in its non-breeding season. Anoestrus is sometimes the result of a separation between physiological oestrus and behavioural oestrus when the former functions and the latter fails. Complete anoestrus results from ovarian dysfunction due to a variety of factors such as malnutrition, parasitism etc.

### III. COITAL BEHAVIOUR

Many of the components of pairing behaviour and coitus can collectively be described as courtship activities. Courtship activities undoubtedly present themselves in behaviour patterns typical of particular species. In any one species the behaviour pattern of courtship consists of several phases which blend into each other in a specific chain of events. The principal behavioural features of courtship behaviour in sheep, horses, cattle and goats are now seen to be essential for optimum gametogenesis and gametokinesis and thereby form the foundations of maximum fertility in the farm animals.

In the sexually mature male, copulating behaviour becomes orientated and directed so that the female is appropriately covered for intromission to be accomplished. This directional aspect of mounting seems to be acquired by learning, but is shown more positively by male animals which are highly stimulated sexually. Disorientation, both in the approach to mounting and in mounting itself, can be seen in male animals with a low level of libido. Male animals seldom mount the females of species other than their own but stallions

will mount female donkeys and jackasses will, likewise, mount mares. Such inter-species coitus allows mules to be bred. Occasionally sheep and goats will intermate but normal pregnancies do not result. There have been reports of isolated instances of abnormal sexual behaviour in which the male of one species mounts females of a different species. Examples include bulls mounting mares, stallions mounting heifers and dogs mounting various species. In each of these cases the animals involved were stated to have been in each other's company from early life, and this suggests that the "abnormal" behaviour was a consequence of imprinting.

"False mounting" attempts by the male animal are commonly seen in courtship. In these instances dismounting subsequently follows quickly without any forelimb clasp or pelvic thrusting movements. False mounts show that the mechanics of mounting and of intromission are separately controlled. False mountings are to be seen in the mating patterns of the stallion, the sheep and the goat. In the stallion it is believed that some 2 or 3 false mounts are normal before effective mating is achieved.

Following normal mounting, penile intromission is effected, but this is dependent on prior penile erection (Fig. 169d). Erection in the stallion is much less rapid than in the ruminants. (It may be for this reason that "false mounts" are customarily shown by stallions before mating takes place). In the bull, goat and ram there is a more rapid erection and protrusion. In the ruminant species intromission consists only of a single pelvic thrust which is followed by dismounting (Fig. 169e, f, g). In the

in cow bulls. The pubertal age of he buffaloes is from 3-4 years. When they reach maturity there is great tendency to fight and as such it is necessary to put in either a nose ring or nose spring to keep them under control for service for collection of semen artificially or when they are in service paddocks for natural service or when they are to be tethered for exercise on spacious grounds or paddocks. A sexually mature bull will not tolerate the presence of other bull and if they happen to be in close proximity fights would generally ensue, such fights are very common in breeding herds.

In majority of the establishments where buffaloes are reared under stabled conditions, it is customary to present the she buffalo on heat to the buffalo bull. It is not customary to let loose the bull when the she buffaloes are free in paddocks as is the case with cow herds. This is in view of the fighting and more aggressive nature of the buffalo bulls. When the she buffalo is on heat, a buffalo bull is brought in close proximity, when he will show marked ferociousness by pawing the ground with his forefeet, digging the ground with horns, dashing his head against the solid bodies and vizzing and snorting very frequently. Olfactory senses are very well developed and he will not take much time to diagnose if the she buffalo is in proper heat. The pre-stimulation such as licking of urine and vulval lips, brushing the tongue to and fro on the vulva is usually noticed. The he buffalo may rest his head and draw his chin on the croup of she buffalo several times to stimulate her sex desire. Frequent micturition is also noticed.

Buffalo bulls are rather heavy and while mounting it is usually seen that

they are rather slow in lifting their front part of the body high up in the air and balance properly on the rear legs. It is the usual experience to find he buffaloes doing faulty mounts. In such cases they are unable to reach the she buffalo and the erect penis cannot properly come in contact with the vulval aperture. However, with practice the bulls get used to proper mounting and the service from such experienced bulls with high sex libido is usually very quick.

The holding or securing the she buffalo by their fore legs after mounting is not very firm as that in cow bulls. The penile movements to locate the vulva are bit sluggish as compared to cow bulls.

The thrust is not so powerful as in cow bulls although the movements of lumbosacral region, muscles of the hind limb, rigidity of the tail and muscular tremors are very well marked than that in cow bulls. Buffalo bulls have a tendency to show better reaction after wall-owing or after a cold water shower.

After the thrust act with ejaculation there is marked exhaustion and it will be observed in majority of he buffaloes that they will loose all their ferociousness and lie resting with their head and forelegs on the back of she buffaloes. In a number of instances they are actually required to be pulled away.

Younger bulls and bulls with high endocrine constitution are usually ready in about 8-10 minutes time for the second service.

Due to seasonal trends in breeding, the males are more active during the breeding season and render natural service to one or more buffaloes on the same day and frequent services at short intervals during the entire season. Un-

Tab  
COITUS IN DOMESTIC UNGULATES

Species	Male Reaction Time	Pre-coital Behaviour of male	Manner of Intromission	Approximate duration of Intromission	Repeat Matings	Ejaculation Characteristics	Remarks
Bovine	mode 2 mins. mean 12 mins. mean of beef breeds 20 mins.	Noses vulva. Genital olfactory reflex. Alignment. Licks hindquarters	A single pelvic thrust co-ordinated with clasp reflex.	5-10 secs.	Free ranging bulls will serve cows 3-10 times in the heat period.	A single ejaculation (rarely two) into vaginal fundus.	Pre-coital behaviour sometimes erroneously termed "courtship". In genital olfactory reflex the neck is extended, head raised, upper lip upcurled, nares constricted. In the clasp reflex the forelegs are strongly drawn backwards and inwards.
Equine	Averages about 5 mins.	Noses genital region. Genital olfactory reflex. Bites croup region. Penis erects fully.	1-4 mounts. Several pelvic oscillations. A terminal inactive phase.	1 min.	Breeding usually arranged to permit 3 or 4 services per heat.	Numerous pulsatile ejaculations directly into the uterus. (In most cases).	Stallion capable of breeding several times per day throughout breeding season (Spring).
Porcine	1-10 mins.	Approaches sow giving series of grunts. Noses vulva vigorously. Champs jaw and froths at mouth.	Short protrusions of spiral penis repeated till intromission occurs. Pelvic oscillations followed by somnolent phase.	9 mins.	Many boars will serve a sow 3-7 times in a heat period.	Prolonged ejaculation into lumen of cervix/uterus. Testes are retracted during seminal emission.	Libido declines during day in hot weather. Poor clasp reflex.
Ovine and Caprine	0.5 to 5 mins.	Noses vulva. Genital olfactory reflex. Paws with fore foot. Bleating, stamping with fore foot, rapid licking. Genital olfactory reflex.	A very quick single pelvic thrust with fore limb claspings.	5 secs.	The buck and ram will sometimes serve oestrous female several times. Some mature rams will serve each ewe only once.	Single ejaculation into vaginal fundus and region of cervical orifice.	Urethral process of penis of ram spreads ejaculate about. Libido in both species declines in non-breeding season which is the period of more light than dark per day.



Table 2  
OESTRUS AND COITUS IN THE DOG AND CAT

Subject	Ave Duration	Vagina/Vulva	Behaviour	Ovulation/ Ejaculation	Remarks
<b>DOG</b>					
<u>Proestrus</u>	8-12 days	Hypertrophied labia Sanguineous discharge	Non specific Consorts with males but avoids mating attempts	Graafian Follicles mature as a crop (up to 20)	Emits odour attracting males over some distance
<u>Oestrus</u>	6-9 days	Hypertrophy reduced Vulva relaxed and dependent Clear mucus	Permits repeated matings throughout entire period	Spontaneous ovula- tions occur within first 4 days of period	First oestrus may occur 8-10 months of age and recur bian- nually without marked seasonal incidence
<u>Coitus</u>	2 mins Intromittent movements 1-20 mins tied	Muscular ring of posterior vagina contracts behind bulb of penis	Bit h sty cnary during mating Eventually moves and breaks tie	Intravaginal ejacula- tion commences in phase of pelvic thrusting and may continue into part of tied phase	As penis relaxes male steps over bitch and 'tie' continues back to back Gestation=63 days
<b>CAT</b>					
<u>Oestrus</u>	3-10 days	Vulva visible Clear mucus in small quantity	Frequent vocalising (espec in Siamese) Restlessness rolling Elevation of tail Exhibits lordosis treading and tail quivering when back is touched	6-8 follicles mature Ovulation occurs only in response to coital stimuli	Cat may seem ill to owner Estrus commonly recurs 2-3 or more times in each breeding phase Estrus common while nursing litter
<u>Coitus</u>	3 secs.	Tilted towards male	Male bites neck of queen treads arches backs and effects single intromission queen then emits cry and turns on male	Small single intravaginal ejaculation	Several matings likely to occur Gestation = 62 days

# Chapter 53

## Effect of stress on reproduction

Reports on the effect of various types of stress on the reproduction of animals are very few. Modernization including mechanization of husbandry practices with the motive of increased production, undoubtedly, impose increased stress on the animals. It is also well established that the tropical climate pre-disposes the animal to severe stress and strain. With more emphasis being laid on cross-breeding programmes in recent years, this aspect requires a thorough study since the progeny of the exotic sires of temperate zone is bound to be stressed under tropical conditions. Except for studies on general adaptation of exotic animals and their crosses to tropical climate, no concrete research work is traceable on the effect of stress on reproduction.

Selye (1950), has thrown much light on the phenomenon of General Adaptation Syndrome (GAS). During the last two decades the Scandinavian workers have shown that the predisposition of endocrine balance is hereditary and due to the various stress factors result in hormonal imbalance having further impact on production and reproduction.

Stress means pressure or tension. Animals are put to stress under extreme domestication. Malnutrition causes

stress to the animals. Each organism has to fight against stress of common occurrence viz. excessive cold, heat, burns, injury etc.

When in health various organs of the body do their functions in a coordinated manner. Beating of the heart, movements of intestines, secretion of glands go on so smoothly that one never feels their existence. The coordination of various functions is due to some force, may be called as vital force or life force. When there is any interference in the smooth function of the vital force, the body denotes it by pain, inflammation etc. or due to the damage caused by the interference. There comes some defensive mechanism which tries to remove obstruction. Thus the interference may be called as stress which is shown by the interaction of damage and defensive actions of the body. The cause of stress is called as stressor agent.

Stressor agents are of two types— which cause systematic stress and stress to extensive region in the body. These are non-specific agents which are devoid of ability to act selectively upon certain tissues or organs. The agents which act selectively on certain organs or tissues are specific stressors. It is observed that various non-specific stressors

ance to the stressor is increased and the organism acquires adaptation by way of nervous and endocrine control.

(c) Stage of exhaustion: It is a stage in which body is unable to resist the stressor and hence yields to it. Symptoms of stage of resistance disappear and again reaction like that of alarm stage reappears.

The G.A.S. as described above is generally complicated by specific actions of the stressor. Every non-specific stressor has some or the other specific action also. Anaesthetic will have specific action on nerves, diuretics on water metabolism and so on. Only non-specific damage and defense are the integral part of G.A.S. and hence in a way specific actions modify the course of G.A.S.

The formation of antibodies against foreign proteins, allergic reactions are

specific ones and therefore, have no connection with G.A.S.

The control of stress is done through nervous and endocrine mechanism. Blood pressure, body temperature, glycaemia, osmotic pressure and tissue dehydration and serologic defenses through their respective regulators—nervine and endocrine—are geared up according to the necessity. The stressor causing hypothermia automatically induces the regulator for body heat to induce hyperthermia and thus try to establish normalcy in body functions. But sometimes it so happens that stressor being powerful affects entire body (non-specific stress) and then the sub-centres of the above named functions cannot maintain normalcy and hence cause central centres situated in hypothalamus and hypophysis to regulate the whole affair. It is here that the general adaptation syndrome comes into action and gear the whole body towards defense.

Sub-centres for various defenses act as given below:

#### *Mode of action*

1. Blood pressure	—	Through endocrine-adrenal cortex and medulla-kidney.
2. Body temperature	—	Adrenal medulla, through action of vasomotor and vasodilator nerves.
3. Glycaemia	—	Through glucocorticoids and adrenargic compounds.
4. Osmotic pressure and tissue dehydration	—	Through mineralo-corticoids which influence electrolyte composition of blood and permeability of membrane.
5. Serologic defense	—	Through corticoids which stimulate the elements of reticulo-endothelial and lymphatic system.

Each of these sub-centres receives specific impulses from its respective target organ. These messages enable the centre to adjust its action so that stability is maintained.

G.A.S. is controlled by two ways. 1. Nervous and 2. Endocrinal. Hypothalamus is the centre of nervous control and hypophysis is the centre for humoral control. It is not exactly known as to

cause adrenals to secrete more salt retaining hormones. This leads to increased concentration of sodium ions in the follicular fluid and subsequent degeneration. Cows which have cystic ovaries have got hereditary predisposition and hence such animals are not good from breeding point of view.

Milk fever is another condition where immediately after parturition the animal is put to stress due to large amount of calcium required for producing milk. High milking animals which can not mobilize the calcium reserves in bone will show typical symptoms of milk fever due to sudden stress. This can be cured by calcium injections which will increase blood level and keep the same till the body adopts to this condition by mobilizing calcium reserves.

Adaptation diseases are divided in three groups :

1. Diseases due to hypo-adaptation.
2. Diseases due to excessive adaptation.
3. Diseases due to abnormal adaptation.

## I. Hyper-functional and dysfunctional

- (a) Primary diseases of organs of adaptation.
  - (i) Cushing's disease (pituitary hyper-function).
  - (ii) Adrenal tumour with Cushing's syndrome (Adrenocortical hyper-function).
  - (iii) Chromaffinomas (adrenal medullary hyper-function).
  - (iv) Primary diseases of kidney conducive to hypertension (Renal hormonal hyper-function).

- (b) Secondary diseases due to excessive response of organs of adaptation.
  - (i) Some types of hyper-tension.
  - (ii) Peri-arteritis nodosa.
  - (iii) Nephrosclerosis.
  - (iv) Rheumatic disease.
  - (v) Some types of appendicitis, tonsillitis, diabetes.
  - (vi) Gouty arthritis.
  - (vii) Various psychosomatic disorders.

## II. Hypo-functional

- (a) Primary diseases of organs of adaptation.
  - (i) Simmond's disease (pituitary hypofunction).
  - (ii) Addison's disease (Adrenocortical hypo-function).
- (b) Secondary diseases due to insufficient response of organs of adaptation.
  - (i) Secondary shock (relative hypocorticism).
  - (ii) Acute gastro intestinal erosions.
  - (iii) Water house (Friderichsen syndrome).

The study of the phenomenon of stress on reproduction has to be basically on the footing that each individual, be man or animal, is a product of heredity and environment. Hereditary make up primarily decides the proneness of an individual to various kinds of stresses. Since the successive events in reproduction of an individual are controlled by a delicately balanced hormonal system, any factor that derails the reproductive process should primarily affect the endocrine balance. Recent studies have shown that the stress pheno-

menon is also a multiglandular syndrome affecting the adrenal cortex, hypophysis, thyroid and gonads. Hence the effect of stress on reproduction is also a multiglandular syndrome involving the adrenal-pituitary-gonad axis. The hereditary predisposition for weak endocrine constitution is already mentioned should apparently affect more pronounced stress effect in our cattle. But the experience in practice is contrary, the effect being largely on production.

There is general agreement that in extremes of climate and inadequate nutrition animals are stressed with impairment of reproductive functions. The effects of stress on reproduction can now be considered under the following heads:

and rams. Rams and bulls poorly adapted to hot climate experience temporary infertility when put in hot weather. Experimental observations that rams kept at 8°C in summer months exhibited higher fertility than their counterparts which were not cooled prove the effect of temperature stress on male fertility (Hafez 1962). Factors which affect the scrotum such as elevation in local scrotal temperature associated with vascular damage, direct thermal damage, blow fly strike, insulation, orchitis, dermatitis and wounds may also be responsible for disturbed spermatogenesis and maturation process. Hypothermia may also adversely affect male reproduction though to a much less severe degree than hyperthermia. Bulls exposed to frost bite produced semen of poor quality (Faulkner *et al* 1967).

to high ambient temperature. Similarly calves under continued heat stress attain maturity later than their counterparts in temperate regions. In Indian dairy animals—especially buffaloes, hot-dry weather of summer produced lowered fertility in terms of conception rate, suppression of oestral signs (Roy *et al*, 1968) and/or cessation of ovarian cyclic activity (Goswami and Luktuke, 1965; Roy *et al*, 1972; Kaikani and Hukeri, 1968).

## Nutritional Stress

### 1. IN MALES

Under feeding in male farm animals, in broad terms, leads to retardation of sexual maturity in young stock and lowering of semen quality in adult individuals. Mann and Rowson (1956) showed that appearance of citric acid and fructose in seminal fluid and appearance of first sperm were delayed by 4 months in an underfed bull calf in comparison to its identical twin mate maintained on normal diet. In ram lambs maturity as judged by separation between penis and prepuce and spermatogenesis is delayed in nutritional stress. In adult animals total energy insufficiency may not affect spermatogenesis, while protein deficiency and deficiency of vitamin A adversely affect sperm quality and sex drive. Prolonged vitamin A deficiency causes severe degenerative changes in the seminiferous epithelium leading to complete lack of sperm production; this defect is corrected in the adults by replacement therapy.

### 2. IN FEMALES

Under-feeding in female growing stock, causes delayed puberty. Underfed adults fail to manifest early oestrus or may show weak oestrus with irregular

cycle length. In sheep, "quiet ovulations" especially in the beginning of breeding season is believed to be due to underfeeding because of forage deficiency. Underfed ewes have reduced ovarian activity as evidenced by reduced follicular number. The precise mechanism of the effect of under-nutrition on ovarian activity is unknown, but it is supposed to be akin to a pseudohypophysectomy effect (Hafez, 1962). Protein deficiency delays maturity and growth and development of genitalia (infantile) in heifers. These effects can be avoided by providing balanced rations. Vitamin A deficiency causes disturbances in foetal development and viability of the offspring. It causes severe damage to the germinal epithelium of testes in the males. Among other nutritive factors, deficiency of copper, cobalt (in ruminants) and phosphorus causes ovarian dysfunction and delayed maturity.

## STRESS OF HIGH ALTITUDE

High altitudes above 14,000 feet above sea level may have adverse effect on testicular function and fertility; but conclusive proofs are lacking.

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# Chapter 54

## Neoplastic Diseases of the Female Genital Tract

The tumours of the female genital tract are not uncommon.

### OVARY

Ovarian tumours occur in all animals but are most commonly seen in cows and bitches. According to their origin, they are classified by Nielsen *et al* (1976) as:

1. Epithelial tumours—Adenoma and Adenocarcinoma.
2. Sex Cord—Stromal tumours—Granulosa Cell Tumour, Theca Cell Tumour and Luteoma.
3. Germ cell tumours—Dysgerminoma and Teratoma.

#### Adenoma and Adenocarcinoma

These are among the commonest of the ovarian tumours of the bitch and have been recorded in other species as well. A majority of these are serous and adenomatous or carcinomatous though sometimes they are mucinous. They originate from the germinal epithelium of the ovary and project on the surface as cystic nodular growths. They may be unilateral or involve both the ovaries and their cut surface presents numerous cystic cavities separated by fibrous stroma.

Histologically, the tumour reveals the usual feature of an adenoma or adenocarcinoma comprising of acinar structures lined by a cuboidal epithelium. The latter is often thrown into papillary folds projecting in the acinar lumen which contains a pale eosinophilic fluid. The lumen of the acini may get obliterated in the form of rapid growth. Connective tissue stroma is moderate.

Adenocarcinoma can be identified from adenoma by its invasive tendency, hyperchromatism of the cell nuclei and mitotic activity.

#### Behaviour

The malignant tumour invades the ovarian bursa and on gaining access to peritoneum, causes secondary growths across the peritoneal cavity on the visceral organs.

#### Granulosa Cell Tumour (Fig. 170, 171)

A common tumour of the ovary, in most of the domestic animals, and is frequently encountered in cows, bitches and mares. They attain a large size and Cordes (1969) recorded tumours in equines attaining a diameter of 23 cm. This tumour is of par-



ticular significance in view of the hormonal disturbances it evokes

Usually unilateral, the growth is lobulated, grey in colour, solid or cystic and possesses a well defined capsule.

Histologically, the tumour parenchyma is made up of uniformly round or oval cells resembling the granulosa cells of an atretic Graafian follicle. They may be arranged diffusely or may present a pattern resembling developing follicles. Hyaline proteinaceous material is at times present in the spaces enclosed by these cells simulating the morphology of the ovum. These are the "Call Exner bodies" described in human granulosa cell tumours.

Some of the granulosa tumours reveal a pattern similar to that of sertoli cell tumour of the testis and then the cells are oval and arranged in a tubular fashion.

Luteinization of the tumour is not uncommon. Mitotic figures are usually scanty and connective tissue stroma is also poor.

### Behaviour

The main feature of this tumour is the feminization syndrome that results from the oestrogens elaborated by the tumour cells. This has been observed in all animals and is characterised by nymphomania and cystic endometrial hyperplasia. In the bitches, uterine tumours and pyometra often accompany this symptom complex.

If unilateral, removal of the affected ovary causes the symptoms to recede and fertility may be restored.

### Dysgerminoma

This is a rare tumour of animals arising from the undifferentiated cells of the ovary. It has been recorded in

the cow and the bitch (Smith *et al*, 1972). Solitary cases have been reported in the bitch by Taylor and Dorn (1967), Buergelt (1968), Vaidya and Ajinkya (1969), Ishmael (1970).

Grossly the tumour is soft and usually presents a lobulated appearance. Histologically it mimics the appearance of the testicular seminoma and hence is sometimes also referred to as "seminoma of the ovary". It is made up of a fibrous stroma separating alveolar or tubular structures which enclose clusters of large, round or polygonal cells.

### Teratoma

The features have been described in testicular tumours. The cystic variety of the dermoid cyst has been reported in buffalo heifers (Kodagali, 1979).

### Thecoma

Thecomas are rarer than other ovarian tumours and consist of fusiform or spindle shaped cells showing an arrangement of interlacing bundles. They are sometimes associated with hyperoestrogenism, otherwise they are benign and remain confined to the ovary.

nant tumour of the canine uterus. In contrast to the situation in women, cervical carcinomas are very rare in animals.

## Adenocarcinoma of the uterus

### INCIDENCE

**Cattle:** This is a most common uterine tumour in this species, occurring usually in animals over six years of age. A number of cases have been reported by Monlux *et al* (1956) and Brandly and Migaki (1963). It is considered by these workers to be of great economical importance and its frequency in cattle is next to ocular carcinoma and lymphoid leucosis. The tumour has not been reported so far in India.

In other species of animals, this tumour has been rarely reported. Isolated cases have been reported in Sheep (Terlecki and Watson, 1967). Goat (Brandley and Migaki, 1963). Sow and bitch (Joshi *et al*, 1967) and Cat (O'Rourke and Geib, 1970).

### Histogenesis

The tumour arises from the endometrial lining or its glands.

### Gross appearance

Usually the tumour is inconspicuous at the gross examination and may escape attention. It causes a local thickening of the uterine wall and according to Monlux *et al* (1956) it produces annular constrictions at the site. On incision, the growth appears as sessile, firm and yellowish grey nodules, embedded in the uterine wall. The usual site is the cornua and only rarely the corpus is affected. Histologically, the tumour shows the usual features of an adenocarcinoma, being composed of irregular

tubular structures lined with columnar cells. The tubule lumen contains much debris and exfoliated cells. Invasive tendency is very much marked and involves all the uterine wall elements viz. endometrium, myometrium and the serosa. The tubular structures are separated by a considerable amount of fibro cellular stroma. In the bitches and the cat, however, the stromal element is usually less conspicuous.

### Aetiology

Prolonged hyperoestrinism has been incriminated in the causation of uterine tumours in man and animals. This is because in many cases the uterine tumours are associated with follicular cysts, endometrial hyperplasia and mammary gland hypertrophy. Interestingly enough, in the bitch, though follicular cysts and endometrial hyperplasia are common, they most often lead to pyometra and rarely the uterine tumour. Prolonged progesterone phase in the oestrous cycle of the bitch has been cited as one of the possible causes of rarity of uterine carcinoma in bitch vis-a-vis in women.

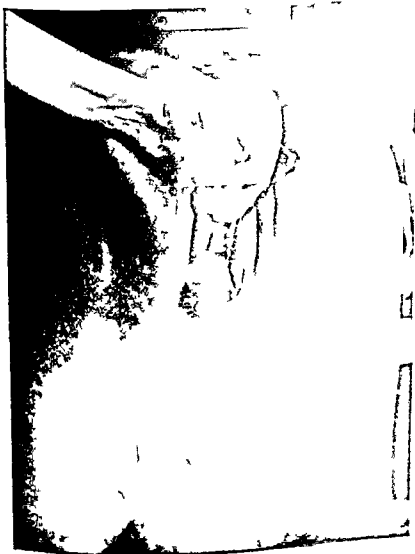
### Behaviour

No clinical symptoms are shown by the affected animal and Monlux *et al* (1956) observed no adverse effect on the fertility of the animal. Normal delivery can occur in the tumour bearing animal.

The adenocarcinoma is however highly invasive and penetrating through the uterine wall, the tumour spreads through the peritoneum to cause secondaries on the serosa of abdominal viscera. Distant spread via lymphatics and blood vessels is common in sublumbar and iliac lymph nodes as well as lungs and its lymph nodes.



Fig 170 Granulosa cell tur



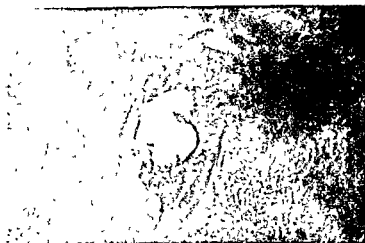


Fig. 173 (a) Vaginal polyp in a buffalo.



Fig. 174. Carcinoma of vulva involving right labium.



Fig. 173 (b) Vaginal polyp in a buffalo  
Note the pedicle



Fig. 175 Carcinoma of vagina in the swine.

## Leiomyoma

This is the commonest tumour of the tubular genitalia of the bitch and occurs in the uterus and the vagina. As in the Adenocarcinoma the usual site is the uterine cornu, though other parts may also be involved. This tumour is commonly designated as uterine fibroid owing to a substantial participation of collagenous stroma in the formation of the tumour. Next to bitches the tumour is common in cows (Kronberger 1961 and Sane *et al* 1958) (Fig 172). Isolated cases have been recorded in other species.

### Gross appearance

These tumours may be solitary or multiple and occur as round or oval pedunculated or sessile greyish white nodules hard in consistency in the lumen of the uterus or of the vaginal passage. The consistency may however vary according to the extent of participation of muscular and fibrous elements. The cut surface is lobulated and shows whorl-like arrangement. Due to haemorrhage or necrosis brownish or blackish discoloration may also be observed. Histologically the tumour parenchyma is seen to be made up of parallel and interlacing bundles of plain muscle fibres. Transversely cut bundles show a whorl-like pattern. The muscle cells are spindle-shaped, plump and possess a fusiform and vesicular nucleus. Mitosis is rare. Vascularity is also moderate. The connective tissue component is variable in different regions of the tumour and is collagenous in nature.

### Histogenesis

The growth arises from the smooth muscle fibres and the connective tissues of the affected part.

## Behaviour

These tumours are usually benign and rarely cause any clinical or functional disturbance. When malignant which is rare it may invade locally.

## CERVIX

The neoplasms of bovine cervix are very rare and usually benign. They include fibrosarcoma, leiomyoma and carcinoma. Adenocarcinoma may become malignant and metastasize to other visceral organs. These have been described by Wadsworth (1952), Cotchin (1956) and Moulton (1961). Squamous cell carcinomas of the cervix are uncommon. Anderson and Sandison (1969) have recorded three cases in a survey of tumours of bovine female genitalia in the British Abattoir.

## VAGINA

Fibromas, fibrosarcomas, leiomyomas, haemangiomas, lymphosarcomas and carcinomas of bovine vagina have been reported by Williams (1943), Wadsworth (1952), Cotchin (1956) and Moulton (1961). They are very rare and if large may lead to dystokia. During calving they are liable to be torn and invite infection. The tumours are usually benign, pedunculated and protrude through vulva when the animal is recumbent (Fig 173a, b). The most common tumour is fibroid with a tachement at or near the hymenal ring. Surgical intervention by ligating the pedicle may be necessary. Sometimes the tumour may be extensive and excision may be difficult. Such a removal may weaken the vaginal walls and lead to herniation of other organs as a post-operative complication. These tumours rarely account for infertility in bovines.

The malignant tumours in bovine vagina and vulva are rare. Squamous cell carcinoma of the vulva has been reported by Burdin (1964) in 6 Ayreshire herds in Kenya. In four of these herds 29% were found to be affected. The incidence in the non-pigmented and partially pigmented vulvas was 55.5% and 38.3% respectively. Significantly no case was found in Sahiwal breed which had pigmented vulvas. He suggested that the tumour induction was due to solar radiation.

The tumours arose as acanthosis and papillomas but later became carcinomatous. A solitary case of carcinoma of the vulval lips in a Gir cow has been recorded by Sane (1978). The cauliflower like growth was operated upon twice but there was recurrence (Fig. 174). Though this did not affect fertility, the foetid discharge always soiled the perineum and the udder and lead to infective condition of vulva and udder.

Carcinomas of vagina and cervix have been reported in swine (Fig. 175) by Monlux *et al* (1956). In dogs and cats, benign tumours of cervix, vagina and vulva include leiomyoma, fibroma, neurofibroma, fibroleiomyoma and lipoma.

These have been described by Bloom (1954), Cotchin (1956), Moulton (1961), Brodey (1968) and McEntee (1970). The malignant tumours are very rare and include carcinomas, fibro-sarcomas, sarcomas, chorio-epithelioma of placenta and hydatidiform mole in cat (Riser, 1940; Bloom, 1954; Cotchin, 1956; Moulton, 1961).

### MAMMARY GLANDS

Like the transmissible venereal tumour, the neoplasms of the mammary gland present some interesting features.

### Aetiology

It has been observed that the incidence of mammary tumours is usually seen in conjunction with disease conditions of the ovary and the uterus. These include follicular cysts, persistent corpus luteum, cystic endometrial hyperplasia, pyometra and uterine tumours. It thus appears that there is some casual relationship between the two conditions. These tumours are also seen in animals which have not bred for long; in those with irregular oestrus; pseudo-pregnant lactating and in those which have failed to suckle the young (Cotchin, 1958). Little (1927) visualises the development of mammary tumours as related to mastitis following improper milk drainage. Dystokia and death of litter were also suggested as contributory factors (Cotchin, 1956). Jabara (1960) considered that there is a hereditary predisposition. Backman (1945) observed a tendency towards mammary carcinogenesis in three generations of Boston Terriers.

Mulligan (1949) feels that an abnormal development of cell rests is responsible. However, as Allen (1940) has pointed out, if this was true, the tumour should develop in males also with equal frequency. Cotchin (1956) feels that the caudal glands in canines are more subject to trauma, are larger and more actively lactating and therefore more prone to development of tumours.

It must however be conceded that oestrogens play an important part in mammary carcinogenesis. That hyper-oestrinism is a factor to be reckoned with is evidenced by the rarity of tumours in bitches spayed at an early age (Cotchin, 1956 and Moulton, 1961) and regression of the already existing tumour after ovariectomy (Meier, 1963). Sastry and Tweihouse (1964) observed some mam-

mary tumours to be associated with adrenal tumours and as Robbins (1959) has pointed out in old age adrenals produce oestrogens

In this context it is interesting to remember that in mice where a virus Bittner Milk Factor has been found to be an aetiological agent for mammary tumour development the incidence declines after ovariectomy even in those strains which are highly susceptible to the action of the crustative virus

### **Incidence**

In domestic animals the mammary neoplasms are commonest in the dog in which species it is the tumour which ranks first in occurrence of various tumours

### **CATTLE**

Despite the fact that the mammary gland has reached its maximum development and has attained a high degree of functional efficiency in the bovines this gland is rarely the site of neoplasia in cattle (Moulton 1961). Quite a large number of cases reported are in fact the tumours of the skin of the udder which have invaded the gland parenchyma

In Indira Nair and Saxty (1954) recorded only one case of carcinoma that had developed from the milk duct

### **HORSE**

The equines are only infrequently the subject of mammary tumorigenesis (Feldman 1932) though the incidence in this species is rare than in bovines

Cunningham (1903) reported a case in which the growth was considerably large and had metastasised to the lungs. In France Martel (1913) reported 45 cases of mammary tumours amongst 39 800 horses slaughtered for food

### **SHEEP AND GOATS**

There are no records of mammary neoplasia in either of these species indicating the extreme rarity of this condition

### **Pig**

Only isolated cases are on record. They are reported by Allen (1910) Wankmuller (1902) Schrieble (1932) and Feldman (1932)

### **Dog**

From the observations of various workers it is estimated that in general the incidence of the tumours of mammary gland varies from 25 to 40%. Sticker (1901) encountered 333 tumours of this gland amongst 739 canine tumours. Corresponding figures given by other workers include Cotchin (1959) 89 out of 1086. Smith and Jones (1961) 931 amongst 5851. Mulligan (1949) 23 in 120. Joshi (1966) recorded 2 mammary tumours in a study of 77 tumours in dog. Saxty (1959) recorded 10 tumours in a study of 320 tumours. At the Department of Pathology Bombay Veterinary College of the 730 canine neoplasms studied 228 were accounted for those of the mammary gland

Mulligan (1949) reported that certain breeds such as Cocker spaniel, Fox terrier and Boston terrier are especially predisposed to the development of these tumours. However Jabara (1960) denies such a predisposition

The mammary tumours are observed almost exclusively in the female though they are occasionally seen in the males (Cotchin, 1951, 1956).

It is universally observed that the tumours occur in middle or old age. Cotchin (1951) noticed that 151 of the 187 mammary tumours were from animals over 8 years of age. The incidence is said to rise with age.

Of the five pairs of glands that are present in the dog, the caudal three are mostly affected and account for 60% to 80% of the mammary tumours (Fig. (176a, b). According to Runnels *et al* (1960), the third gland is the least affected.

## CATS

Cats rank next to dog in the frequency of mammary tumours (Moulton, 1961). Murray (1968) encountered four mammary carcinomas in 11 feline tumours studied. Sticker (1902) came across 5 tumours of this kind amongst 21, while Field (1894) reported two cases in 15 year old animals, one of which showed a generalised metastasis. Smith and Jones (1961) reported 11 tumours of mammary parenchyma amongst 174 tumours from this species. Solitary cases have been recorded by Rousay and Woit (1920) and Petit (1903). Mammary tumours have been described in castrated males occasionally (Moulton, 1961).

In general, the mammary tumours are of three types as described below.

### A. Duct Papilloma (Papillary Adenoma)

**Gross appearance:** This tumour arises as solitary or multiple circumscribed lesions from the gland ducts. They may be sessile or pedunculated and their consistency may be cystic or firm.

## HISTOLOGICAL APPEARANCE

It is made up of ductlike structures lined by a single layer of cuboidal epithelium resting on a variable amount of collagenous tissue. Depending on the rate of proliferative activity and secretory potential, papillary projections and accumulation of an eosinophilic homogeneous material in the lumen are discernible. At times the connective tissue may show a tendency to myxomatous or cartilagenous transformations.

### B. Adenocarcinoma

This is the commonest mammary tumour in the cat.

## GROSS APPEARANCE

Displaying a great variation in size, these malignant tumours often attain a considerable size. They may be round, oval or flattened and generally ill circumscribed. They are infiltrative and therefore lack a stalk or a capsule. They may be either hard (scirrhous), soft, spongy or cystic.

## HISTOLOGICAL APPEARANCE

Typically, this tumour shows widely invading acinar structures separated by differing amounts of stroma. The normal histological architecture of the gland is considerably altered. In rapidly developing tumours, the cells tend to form solid masses of cells which may be devoid of a lumen. The tumour cells are then seen to infiltrate adjacent tissue. In a well differentiated tumour, papillary folds of the acinary surface and evidence of secretory activity are noticeable.

The tumour cells are cuboidal or columnar, possessing a large round or oval hyperchromatic nucleus. Mitotic figures are variable in number. The



stromal connective tissue may show inflammatory reaction.

### C. Mixed tumour or Terratoid Mixed Tumour

#### GROSS APPEARANCE

This is most commonly seen in dogs but is rare in cats. These tumours greatly vary in size and consistency and may weigh upto 500 gm. They are encapsulated and freely movable. Lobulation and cyst formation are common.

#### HISTOLOGICAL APPEARANCE

The tumour is seen to be formed by epithelial and connective tissue elements and either of them may predominate. The epithelial components form patterns similar to those in adenoma or adenocarcinoma. However in most of the tumours the connective tissue elements overshadow the glandular formation and there is a variable admixture of proliferating myoepithelial cells, developing cartilage or bone tissue and areas of connective tissue mucoid, containing stellate cells. Development of heteroplastic myeloid tissue and areas of cholesterol deposition are the frequent features.

The development of cartilagenous islet is attributed by some to the epithelial elements (Cohrs, 1966), while others trace its origin to the myoepithelial cells (Moulton, 1954 and Willis, 1960).

#### BEHAVIOUR

Both benign and malignant varieties are encountered. Even when malignant, the tumour grows slowly and metastasises late. The frequency of metastasis has been estimated by Krook (1954) to be as high as 50%. It is suggested that the incidence of metastasis increases if animals are allowed to live for a longer time. Metastatic lesions first occur in the supramammary lymph node and later secondaries may develop in lungs and visceral organs.

Oophorectomy in conjunction with mastectomy prevents recurrence in the bitch. However no such effect is seen in the cat.

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# Chapter 55

## Neoplastic Diseases of the Male Genital Tract

A study of the neoplasms of the genital tract is a fascinating one. This is because, frequently, they are associated with endocrine disturbances. In fact, most of the examples cited in support of the hormonal theory of tumorigenesis are from the genital tract. On the other hand the tumours of the Ovaries and the Testis have themselves been shown to produce hormones inciting either masculinising or feminising manifestations. This fact can very well be appreciated if one remembers that the ovary as well as the testis are initially bisexual structures. In the ovary, the outer cortex of the germinal epithelium is the 'female part' while the medulla is the "male part", which is normally suppressed by the former. Reverse is true in case of the testis.

It is interesting to note that, not uncommonly, the tumours of the genital tract are associated with their primary multiplicity. This may take the form of a bilateral involvement of an organ or simultaneous affection of different components of the same system. Further an organ, especially the testis may reveal two or more individual tumours either of the same type or totally distinct varieties. It is generally observed that if a tumour develops in any part of the re-

productive system then the other parts of genitalia are more prone to develop a tumour simultaneously or at a later stage. Under such circumstances the possibility of a common aetiological agent operating may be visualised.

At the Bombay Veterinary College during a study of 30 cases of dogs with primary multiplicity of tumours involving various systems of the body, it was observed that in majority of cases at least one of such tumours was located in the genital tract. Here an aetiological relationship is difficult to think of. More probably it is a reflection of the relative frequency of genital tumours in this species.

All sorts of tumours occur in the genital tract. In the following pages, therefore, only those tumours which have some special significance in the Pathology of genital tract have been dealt with.

### THE MALE GENITAL SYSTEM

#### Testis

Apart from the Teratoma, there are three other varieties of primary tumours arising exclusively in the testis. These are Seminoma, Sertoli cell tumour and Leydig cell tumour.

## Horse

Apart from Teratoma, the testicular tumours are rare in these animals. Some of the few records available in literature are those by Schlegel (1924) who have described 35 cases in horse and three in ass. Kimura (1917) reported 49 testicular tumours in 77,000 slaughtered horses. Metastasis of Sertoli cell tumour in the lung and the mediastinum has also been reported. In India, apparently the only record is the one by Joshi and Purohit (1962, 63) who recorded a Seminoma from undescended testis.

## Sheep and Goats

The tumours of male gonads are uncommon in these species and only a few cases of Seminomas and Sertoli cell tumours have been reported. Benign Seminomas have been reported in seven of the 154 testes examined by Jensen and Flint (1963). Infiltrating seminomas are reported by Shortridge and Cordes (1969) and Watt (1971). Sertoli cell tumour has been described by Shortridge (1962).

## Dog

A large number of reports of testicular tumours are encountered in the literature. Following the earliest record by Sticker in 1902, a number of workers have reported them and excellent reviews of their findings have been made by Cotchin (1960) and Moulton (1963).

From the observations of these workers, it is apparent that the incidence varies from 0.5% to 13%. The tumours occur mostly in animals over six years of age, the usual age being 10-11 years (Schlotthauer *et al*, 1938; Scully and Coffin, 1952; Survashe *et al*, 1967).

In India, only two records are available. Survashe *et al* (1967) reported 20 tumours in 777 tumour bearing cases while Nair and Parthasarathy (1969) reported 8 tumours in 450 tumours studied by them.

There is no unanimity regarding the incidence of various tumour types of testicular neoplasms in the dog. In the Cotchin's study Sertoli cell tumours were the commonest (42.4%) followed by Leydig cell tumour (28.3%) and Seminoma (13.5%). Other workers have observed Seminoma to be the commonest while Leydig cell tumours to be the least frequent, while Moulton (1960) and others share the opinion that Leydig cell tumours are the commonest. In the report on testicular tumours by Survashe *et al* (1967), Seminoma accounted for 9 and Sertoli cell tumours in 7 cases. The remaining four were Leydig cell tumours.

Sertoli cell tumours have also been recorded in male pseudohermaphrodites (Frey *et al*, 1965; Norrdin and Baum, 1970).

## Cat

Scanty literature is available on testicular tumours in cat; this includes one by Schlegel (1924).

## Seminoma

### GROSS FEATURES

In smaller tumours, the testis shows little or no change in appearance and the tumour can be discovered only when the gland is incised (Survashe *et al*, 1967). Such tumours may be 0.5 to 1 cm. in diameter. In other cases the affected gland is visibly altered. It is enlarged to a varying extent and has a nodular appearance (Fig. 178). In extreme cases, the entire gland is replaced by the tumour tissue. More commonly,

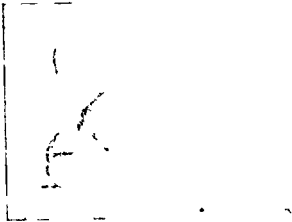


Fig 176 (a) M  
40x



Fig 176 (b) Mammary tumour involving  
3rd right mammary gland



Fig 18 Seminoma of left testicle in  
a dog Compare with the normal  
testicle



Fig 1 (c) Seminoma of left testicle in a bull



Fig 17 (b) Seminoma of left testicle in a bull  
17 (a) c 0.5  
Gouda, Netherland

however the tumour appears as greyish white nodule with a bulging cut surface. Areas of softening and pink discolouration may be noticeable due to necrosis and haemorrhage.

### HISTOLOGICAL APPEARANCE

The histological appearance varies in different stages of development. In early stages the neoplastic cells are confined to the lumen of seminiferous tubules but as the growth continues they break through the basement membrane and spill over into the interstitial tissue. Thus at this stage the tumour cells are seen both in and outside the seminiferous tubules. The growth is usually multicentric in origin but these foci merge together in a well developed growth. The cells then arrange themselves in solid masses separated by delicate and poorly vascularised connective tissue.

The tumour cells are usually large discrete round or oval and possess a moderate amount of pale eosinophilic or basophilic cytoplasm free of lipid material. The nucleus of the tumour cell is large round or oval vesicular and contains a prominent nucleolus. It is hyperchromatic and shows active mitosis. Not infrequently the tumour cells display a syncytial arrangement. Focal lymphocytic and plasma cell infiltration is also evident in some tumours.

### HISTOGENESIS

The tumour arises from the spermatogonia.

### Sertoli Cell Tumour

#### GROSS APPEARANCE

This tumour varies in size and resembles seminoma in its external appearance in being circumscribed greyish white

and firm (Fig 177b). It is therefore difficult to distinguish between the Sertoli cell tumour and Seminoma grossly (Bloom 1954).

### HISTOLOGICAL APPEARANCE

Characteristically the tumour cells show a tubular pattern the tubules being separated from each other by a poorly developed cellular stroma. Because of this feature the tumour is frequently referred to as Tubular adenoma. However in more highly proliferating growths this tubular arrangement is disturbed and gives rise to the one characterised by diffuse cellular proliferation.

In a typical tumour the cells resemble the Sertoli cells. They are large elongated and possess a pale eosinophilic cytoplasm containing varying amounts of lipids. The nuclei are deep stained elliptical and almost of uniform size. In more anaplastic growths the cells may be oval and then they resemble only slightly the Sertoli cells.

### HISTOGENESIS

The tumour cells are derived from the normal Sertoli cells.

### Leydig cell tumour

#### GROSS FEATURES

These are usually small and circumscribed but may grow at times to occupy the entire gland substance. They are yellow brown and may assume a pink discolouration because of the vascularity and haemorrhage. The latter is a common feature of this tumour. The tumour mass is soft and dull and its cut surface is greasy because of the lipid content. The lipochrome in the cells give the tumour its characteristic brown colouration.

### HISTOLOGICAL FEATURES

The tumour is seen to be made up of large, polyhedral cells with an abundant granular or vacuolated cytoplasm containing the lipochrome pigment. In a less differentiated tumour, the cell assumes a fusiform or oval shape. The pale eosinophilic cytoplasm shows the fat droplets which can be demonstrated by usual fat stains. The cells are arranged in sheets or compact syncytial masses separated by sinusoidal spaces containing pale eosinophilic amorphous material or blood. The cell nucleus is large, vesicular, almost of uniform size and round or oval in shape. The mitotic figures are few and it is difficult to distinguish between the benign and malignant varieties.

### HISTOGENESIS

The tumour arises from the normal or hyperplastic interstitial cells of the testis.

### BEHAVIOUR

Testicular tumours usually do not cause any disturbance in fertility unless they attain a large size and happen to occupy a large part of the gland. McEntee (1958) has suggested a possible inverse correlation between the size of the tumour mass, semen quality and fertility. This is especially true of Sertoli cell tumours. Zanwar *et al* (1977) have reported absence of spermatogenic activity in bulls bearing large testicular tumours.

Testicular tumours generally remain localised and metastasis is rare. When present the tumour spread is seen in regional lymphnodes or remote visceral organs (Suryashe *et al*, 1967). This is in contrast to the position in man in which the tumours more commonly tend to become malignant.

Of more common occurrence are the hormonal disturbances associated with these tumours. Seminomas are not known to produce endocrine disturbances (Cotchin, 1960). Leydig cell tumours rarely induce feminising symptoms such as alopecia and precocious puberty (Dozza, 1953; Laufer and Sulman, 1956). In contrast to this Sertoli cell tumours are frequently associated with feminisation. Frequently, the existence of the tumour is first noticed by the attraction to other male dogs. Sertoli cells elaborate oestrogens and therefore the tumour originating therefrom elicit manifestations of hyperoestrinism. This occurs in about 30-10% of the cases (Bloom, 1954; Reif and Brodey, 1969). A significant association between feminisation and Sertoli cell tumours originating from extrascrotal testis has been observed by these workers. When feminisation results the attendant clinical signs are alopecia, pendulous and oedematous penile sheath and atrophy of the other testis free of this tumour. In dogs, prostatic metaplasia has also been observed. These signs regress soon after the tumour bearing testis is removed.

### TERATOMA

These tumours are mixed tumours composed of a variety of tissue types originating from more than one primary germ layers and may therefore be accordingly bidermal or tridermal. It is theorised that the tumour originates from a few misplaced embryonal cells which in early life have escaped the normal process of organisation and later start to grow.

This tumour occurs in both the testes and the ovaries. It is more common in the equine testis but rare in other animals (Moulton, 1961). As in other



testicular tumours, cryptorchidism is a common accompaniment of this tumour. In the dog, ovary is more frequently affected than the testis.

The tumour varies greatly in size and may be solid and cystic. The shape is round or oval. Cystic areas contain viscous material. Some of the cystic tumours contain hair or teeth and are therefore called 'Dermoid Cysts' not to be confused with 'Dermal inclusion cysts' encountered in the skin.

Histologically a teratoma shows an assortment of tissues epithelia of different types, glandular acini, muscles, cartilage, bone and accessory skin glands. Hair, teeth and nervous tissue elements have commonly been encountered (Willis and Rudduck, 1943).

### PENIS

The tumours of this organ are not uncommon. Those of importance are the transmissible Fibropapilloma in the ox, transmissible venereal tumours in the dog and carcinoma in the horse.

#### Transmissible Fibropapilloma

This exclusively occurs in the bovines.

#### INCIDENCE

This is common in some parts of Europe and some parts of United States and is seen mostly in young bulls. In male genitalia is less commonly involved and the growth is restricted to the vagina or vulva. In the bull, the tumour is seen on the glans penis or the prepuce and its neighbourhood.

#### ARTIOLOGY

It is considered that the tumour is of viral origin and is transmitted from one

animal to the other. An experimental proof to this effect is lacking (Cotchin, 1956). However, McEntee (1950) was able to produce this tumour with the virus of bovine papillomatosis. The location of the tumour is suggestive that the transmission takes place through coitus.

#### GROSS FEATURES

The growth is usually solitary and presents a nodular appearance. It is greyish white in colour and has a soft or firm consistency depending on the maturity of the tumour cells (collagenous moiety). It may reach a size of 5 cm in diameter. Ulceration is not uncommon since it is exposed to the exterior.

#### MICROSCOPIC APPEARANCE

The growth reveals itself to be made up of fusiform fibroblastic and collagen fibers forming whorls. The amount of collagen varies in different tumours and according to the degree of differentiation. In young animals the tumour is more cellular and soft whereas in the adults it is more fibrous and hard. The superficial epithelium is hyperplastic so that papilliform projections invade the underlying connective tissue (Pseudoeplitheliomatous hyperplasia).

#### BEHAVIOUR

The tumour remains localized and may invade the adjacent tissues. Spread to distant sites is rare. Recurrence after surgical removal is not uncommon. Formston (1953) observed recurrence in three out of the 12 cases operated. Because of the growth there is a tendency for the bull to bleed during coitus or erection. The animal may be reluctant to serve (Cotchin, 1956; Macdonald, 1951). Regression may occur without treatment (Cotchin, 1956).

## Carcinoma

### INCIDENCE

This tumour is very common in horses and has also been reported in dogs (Cohrs, 1966; Pires, 1944).

### AETIOLOGY

Smegma is thought to be responsible for the production of this tumour (Roder, 1926 cited by Cotchin, 1956). This is supported by experiments in mice by Plaut and Kohn Speyer (1947) to be contributory (Cotchin, 1958). Repeated Trauma and persistent phimosis are also considered to have aetiological significance.

### GROSS FEATURES

The tumours occur on the glans penis or on the preputial lining as a sessile, greyish white cauliflower like growths. Tendency to ulceration may be noticeable.

### HISTOLOGICAL APPEARANCE

The growth is a typical squamous cell carcinoma with central keratinising epithelial "Pearls" and surrounded by several layers of stratified squamous epithelium. Invasion in the surrounding connective tissue is seen at many places. The tumour cell nuclei are deeply stained and show a variable number of mitotic figures.

### HISTOGENESIS

The tumour develops from the preputial lining (Cohrs, 1966).

### BEHAVIOUR

The tumour may metastasise to regional lymphnodes or distant organs like the lungs. Recurrence after surgical removal has also been reported (Barrow, 1895; Neefz, 1923).

## Transmissible Venereal Tumour

This is a peculiar tumour found in the dog which is extensively studied. However, its histogenesis is not clearly understood. Accordingly the growth has been termed variously by different workers.

The tumour arises almost exclusively in the genital tract of the dog. An extragenital occurrence has also been reported (Feldman, 1929; Lacroix and Riser, 1947; Ajello, 1939; Mulligan, 1949). However, unlike the genital growth, these latter growths could not be transmitted to other dogs and hence their true identity remains a matter of dispute (Moulton, 1961).

In the early days, some of the workers disputed the claim that the growth is a true neoplasm. They considered this to be an inflammatory growth (Duplay and Carin, 1894; McFadyen, 1905 cited by Wade, 1908). Hansenmann (cited by Wade, 1908) regarded this to be a mixture of sarcomatous and a granulation tissue. These workers therefore named the tumour as a 'granuloma'.

Subsequently research workers have however emphasised the neoplastic nature of this growth. However diverse opinions have been held by them pertaining to its histogenesis. Wehr (1889) and Geissler (1895) cited by Wade (1908) considered it to be an epithelial tumour and hence called it a carcinoma. Wade (1908) and Smith and Washburn (1898) classified this as sarcoma, since in their opinion it originates from the connective tissue. Among others, White (1902) and Sticker (1901) regard it as a Lymphosarcoma. Beebe and Ewing (1906) termed it as an Alveolar Sarcoma or Endothelioma. Jackson (1911) on the other hand traced its origin to the

Mitotic figures are generally frequent. The growth is fairly well vascularised.

#### TRANSMISSION

Under natural circumstances, the tumour is transmitted through coitus, by implantation of tumour cells in the genital mucosa, and following a slight trauma during coitus (Feldman, 1929). He has also recorded an interesting case in which this tumour was found in a four month old pup involving the right eye and the skin in the frontal region of the head. He attributed this unusual location and the occurrence in so young an animal to a "maternal inoculation" during birth following trauma at parturition.

The view that the transmission occurs by implantation is strongly supported by various experiments in which tumours have been produced by injecting tumour cells in susceptible dogs by subcutaneous intraperitoneal or intravenous routes as well as by implantation, by scarification of skin or genital mucosa (Smith and Washburn, 1898; Geissler, 1895; Wehr, 1889; Novak and Craig 1927; Sticker, 1904; Demonbreun and Goodpasture, 1934; Karlson and Mann, 1952; Bloom, Pfaff and Noback, 1951).

#### BEHAVIOUR

The tumour, though malignant, remains restricted to the genital tract. By local infiltration it may burrow the mucosa deeper. Metastasis, which is not infrequent, is seen only in the regional lymphnodes (Karlson and Mann, 1952; Saxty *et al*, 1965). Occasionally cases of generalised metastasis are reported.

The tumour once it develops remains fixed in its histological features and virulence. Even after several serial implantations, these characters remain un-

altered as was observed by Karlson and Mann (1952) who transmitted it through 40 generations.

After attaining a certain size, the tumour spontaneously commences to regress. This is apparently due to the development of an immunity against the tumour (Demonbreun and Goodpasture, 1934). This is borne out by the fact that the recovered animals are completely refractory to subsequent artificial introduction of the tumour cells in their body (Karlson and Mann, 1952). This immunity is not inherited. It has also been shown that the regression of the tumour can also be effected by injection of blood of a recovered animal in to the affected ones (Crile and Beebe, 1908).

#### PROSTATE

The tumours of the prostate are not uncommon.

#### ETIOLOGY

The causative factors in the development of the prostatic neoplasia are obscure. In man, the hyperplastic and inflammatory conditions of this gland are looked upon as the preneoplastic states. In dogs in which the prostatic tumours are encountered, there does not appear to be a significant relationship between the tumour incidence and these conditions. This is apparent from the fact that in spite of a high frequency of the inflammatory and proliferative changes in this gland in the dog, the tumour incidence is low (Bloom, 1954; Jubb & Kennedy, 1970).

In view of the fact that the normal prostate is influenced by both the androgens as well as the oestrogens, it has been suggested that a disturbance of the equilibrium between these two

tumefaction. Urinary complications are less common and when present, comprise frequent and difficult micturition, haematuria and finally retention of urine. At times uraemic symptoms in the form of anorexia and vomiting are noticeable.

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# Chapter 56

## Procedures in Obstetrical And Gynaec Surgery

Breeding efficiency of female animals largely depends on normal anatomical positions and physiological functions of genital organs. When these two factors are disturbed some form of treatment is necessary. The nature of treatment obviously depends upon the diagnosis of the condition. Besides, some surgical interventions are carried out on an animal which is not suffering from any disease or abnormality with an intention to increase its utility.

### TREATMENT

Prompt attention to such cases is necessary. In delayed cases, it is advisable to allow the laceration to heal and cicatrize. Surgical procedures described by Gotze (1938) for reconstruction of the perineum, can be adopted for all types of such cases with suitable modifications. The operation is conveniently performed in standing position.

## Episiotomy

An incision on the perineum, commencing from the dorsal commissure is at times necessary to widen the passage to vagina. It is particularly necessary in bitches suffering from deep seated vaginal granuloma. In dogs the operation is preferably performed under general anaesthesia. A vertical incision extending up to half of the perineum is taken on the skin and deepened through the underlying tissue. After removal of the neoplastic tissue, the line of incision is closed by interrupted sutures with catgut. The skin incision is closed with mattress sutures using synthetic fibre.

## Laceration of Vagina

Abnormal presentation of digit of the foetus lacerates the vagina more commonly its roof in varying depths. Faulty obstetrical technic particularly while using hooks, chains, ropes etc., is also blamed to cause the laceration. The most common site is vulvo-vaginal border.

Superficial lacerations may not be sutured but deeper ones should be sutured with catgut thread. Post-operative treatment consists of parenteral antibiotics and protective dressing with antibiotic ointments or creams.

## Rupture of Vagina

This condition is characterised by a vent of varying length in the vaginal wall and is caused by malpresentation and improper positions and postures of foetus. Dorso-public position, anterior longitudinal presentation with hind limb extended beneath the body, transverse ventral presentation and when the fore foot or feet are crossed over the neck are the most dangerous positions

of the fetus in this case. Though the condition is reported in all the species of domesticated animals, it is more commonly encountered in mares perhaps due to longer limbs of the foetus.

Forced extraction of foetus in normal presentation pushes perivaginal fat caudally and the vaginal wall ruptures near the hymenal ring or vestibular vaginal border. A similar rupture may occur in cases of prolapse of bladder. Forceful service may cause rupture or laceration of the vagina. Occasional cases are usually met in mares and heifers.

The condition is diagnosed only on vaginal palpation. In most cases no treatment is recommended. The simple tare in vaginal wall heals fairly rapidly. Some workers however recommend suturing of the vent so as to avoid possible complication of perivaginal abscess formation.

## Persistent hymen, Imperforate Hymen (White heifer disease)

Presence of dorso-ventral bands in young heifers is not of great significance as it does not interfere with normal coitus. However, imperforate hymen demands a careful examination and surgical intervention.

The animal will be having regular oestrous cycles. Natural service might cause haemorrhage and straining. Per rectum palpation of genitalia may show distention of a uterine horn due to accumulation of mucus. In some cases vaginal distention and bulging of hymen may be seen. Frequent attempt of micturition is also reported.

Conception may result in the heifers with nearly complete hymen. In these cases dystokia may result and deep episiotomy is the only treatment.

The imperforate hymen is cut open by a crucial incision under epidural analgesia. A circular incision around the vulvovaginal border is also recommended. Gupta and Sharma (1973) treated three cases in buffalo heifers with manual rupture. About 2.5 litres of amber to dark reddish brown discharge was evacuated after perforation of the hymen.

Postoperative care included dilatation of vulvovaginal border with the help of a vaginal speculum. During the operation and insertion of speculum strict aseptic precautions are necessary to avoid introduction of infection which eventually may result in septic metritis.

### Recto Vaginal Fistula

Recto vaginal fistula involves rupture of the dorsal wall of vagina and ventral wall of rectum (Fig 179). In mares recto-vaginal fistula may occur during violent parturition and it commonly occurs in unattended foaling. Immediate attention should be paid to such injuries by promptly putting transverse sutures on the rectal wall. Suturing of the vaginal wall is not necessarily required. If the condition is not detected early, it will be necessary to wait until the oedema subsides and granulation tissue and wound edges heal up before undertaking surgical measures to correct the fistula.

The line of treatment for the recto-vaginal fistula should be on similar lines as recommended by Fowellers cited by Bane *et al* (1963). Epidural anaesthesia by using 2 per cent procain hydrochloride 15-20 ml is given. Back raking should be done to empty the rectum and the perineal area is cleaned thoroughly. A 4-6 inches incision is made horizontally in between the rectum

and vulva. This incision is continued forward 1-2 beyond the anterior border of the fistula keeping as much tissue as possible on the rectal surface. The vent in the rectum is first closed transversely. This will reduce the pressure on the suture line. A Lambert type of suture is used by using heavy non absorbable suture material such as silk or synthetic and removed later. Vaginal tere is sutured on its long axis and skin incision is closed by vertical mattress sutures. Antibiotics are indicated. Heavy concentrate feed should be withheld and bran mash should be given. The bulk should be reduced by reducing the quantities of the hay to avoid load of the fecal matter causing friction on the operated area.

### Rupture of Uterus

In cows and buffaloes the sharp bony prominence on the cranial portion of the pubic symphysis may cut the uterus while the foetus is forcefully extracted. It is due to retraction of the uterine wall. Transverse rupture of the uterus is likely to occur. When a portion of the uterine wall gets itself caught by the deviated extremity it ruptures during forced extraction of the foetus in abnormal position posture and torsion uteri condition. Severe adhesions of the uterus to the abdominal viscera, emphysematous foetus and dry condition of the birth canal contribute to rupture of the uterus.

In the mare rupture of uterus commonly occurs if traction is applied to the foetus in rotated bicornual pregnancy.

In rupture of uterus in the cow or buffalo a variety of symptoms such as restlessness, anorexia, suspended rumination, accelerated pulse and respiration

and cold extremities are usually noticed. There may be rise of body temperature. In case of infective condition of the foetus and uterine contents, septicaemia will set in resulting in peritonitis.

On confirmation of the diagnosis on rectal and per vaginal palpation and symptoms exhibited, laparotomy should be performed to remove foetus and evacuate the fluids. The ruptured portion of the uterus should be sutured by continuous mattress suture. Parenteral and intra-uterine antibiotics should be administered.

Prognosis is usually poor with complications, such as, retained placenta, metritis, atonic uterus and prolapsed intestines.

### Prolapse of Vagina

This condition is more common as compared to other surgical affections of genital tract and is often encountered in cows. Sheth (1970) reported that cases of vaginal prolapse accounts for about 12 per cent of the clinical cases with reproductive disorders in Surti buffaloes.

Irritation of tubular genitalia, laceration or trauma and atony of vagina are the major aetiological factors.

Prolapse may occur antepartum or post partum and may be associated with prolapse of cervix or uterus. In few cases, prolapse of urinary bladder may also be seen.

For the sake of convenience of description the condition is classified in 3 degrees. In the first degree the prolapsed mass is small and is observed as a small donut shape mass extending only up to the lips of vulva, particularly when the animal is sitting. In the second degree

prolapse, vaginal floor or wall protrudes out of vulva; aperture and in the third degree there is complete protrusion of vagina exposing the cervix (Fig 180, 181).

As a result of prolapse, the mass falls over ischial arch and in this position vascular insult causes passive congestion which results in devitalisation of the mucosa. Serum from the submucous oedema oozes out. The mucosa soon undergoes certain ulceration and gangrene ensues in neglected cases. Separation of mucous membrane as well as the submucosa tend to develop into a hard thickened fibrous wall. In cases where gangrene ensues, it tends to progress forward causing gangrene of the cervix.

Treatment includes reduction of the prolapsed mass and its retention in normal position in order to prevent its recurrence. The prolapsed mass is thoroughly cleaned with abundant lukewarm water containing non-irritant antiseptic. Under epidural anaesthesia the mass is reduced to normal position by pushing with open palms. Pressure with finger tips must be avoided. In severe oedematous conditions, the fluid is removed either by pressure with thin cloth or by removing crescent shape strips of mucous membranes and suturing the wound with 3/0 catgut (Farquharson, 1949). With a closed fist at the os uterus the mass is pushed forward.

Various procedures are recommended for retention of the prolapsed portion of the vagina.

### 1. TRUSSES

Application of rope truss had been widely used in the past. This method is useful only in cases with mild tenesmus, loosening and displacement of the ropes may result in failure.



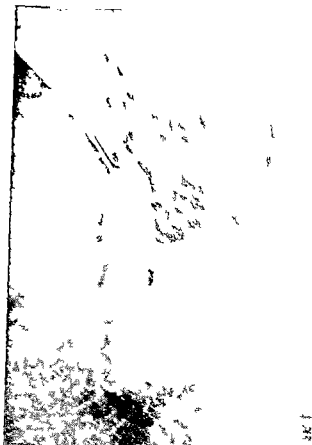


Fig 169 Rectovaginal fistula : a buffalo mare

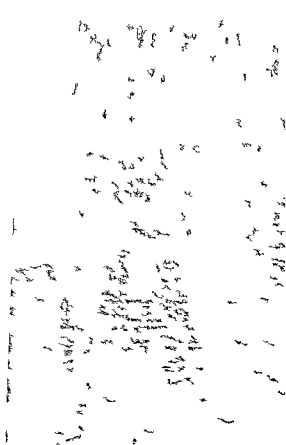


Fig 170 Rectovaginal fistula : a buffalo mare



Fig 181 Vaginal prolapse exposing os uteri in a buffalo

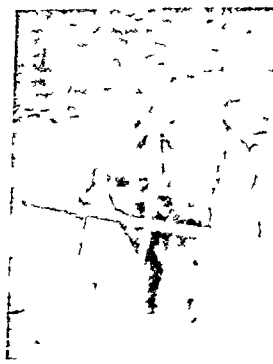


Fig 182 Position of the uterus in a buffalo

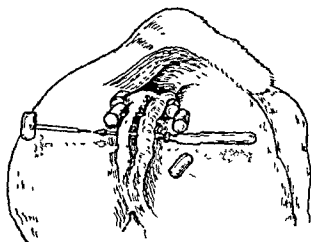


Fig 182 Flessa needle in position

## 2. FLESSA NEEDLES

Under epidural anaesthesia these needles are passed through the lips of vulva slightly anterior to the musculo-cutaneous junction. Depending upon the size of the vulval opening 3 to 4 needles are used (Fig 182). The needles are removed after the cow stops straining.

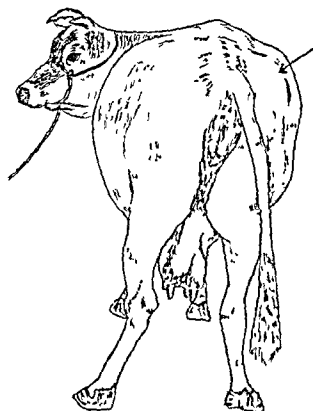


Fig 183 Distension of abdominal cavity on injection of air in peritoneal cavity. Arrow shows the site of injection

(1967) have reported satisfactory results by this method. While passing the needle through the lesser ischiatic foramen care has to be taken not to touch the internal pudic artery and/or the nerve.

#### 6. FARQUHARSON'S TECHNIQUE OF RESECTION OF VAGINAL MUCOSA

It is particularly useful in cases of extensive oedematous swelling of prolapsed mass. Under epidural anaesthesia crescent shaped strips,  $1\frac{1}{2}$  to 2 cm. in width, of vaginal mucosa are removed by submucosal dissection starting from anterior point. Haemorrhage is usually scanty. Wound edges are sutured with 3/0 catgut. Due to escape of fluid the mass becomes smaller in size and the reduction is facilitated.

#### 7. MODIFIED CASLICK'S OPERATION

This operation is reported by Baker (1950) and recommended by Roberts (1971). After reposition of the prolapsed mass, the caudal mucous membrane about 5 to 10 mm. in width and 5 cm. in length, from the dorsal and ventral commissures of vulva is removed, and the lips of vulva are coopted using 28 gauge stainless steel wire (Fig. 185). Since the size of the vulval opening is reduced the chances of prolapse are considerably less. Narasimhan *et al* (1975) reported a technique for retention of chronic recurrent vaginal prolapse by the application of a purse string suture at the region of hymenal ring to prevent vaginal prolapse after its repositioning.

#### 8. PERIVAGINAL FIXATION

Guard (1953) and Buhner (1960) recommend a simpler but efficient method for retention. Under epidural anaesthesia, a small horizontal incision is

taken at the perineum just above the dorsal commissure of the vulva. An umbilical needle or Gerlachs needle threaded with a tape preferably made of synthetic fibre is passed through the skin incision and downward, forward, under the skin and around the vaginal tube, taking care that it does not pierce the vaginal mucosa. When the needle reaches the ventral commissure a small vertical skin incision is taken if necessary and the needle is taken out. The needle is then passed through this opening and pushed upwards from the side so that the other end of the tape comes through the same opening taken on the perineum. The vaginal passage is narrowed by pulling the tape and sailors

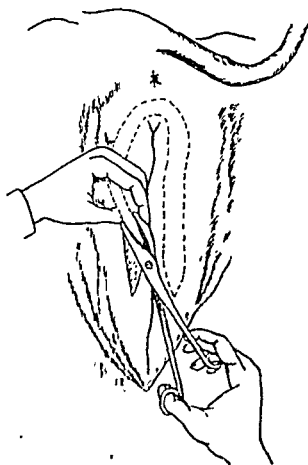


Fig 185 Modified Caslick's Operation for vaginal prolapse. Stripping on vaginal lip. Dotted line shows the extent of stripping

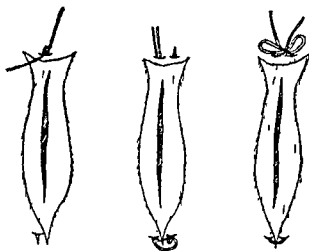


Fig 186 Buhners or Guards method of perivaginal suture

knot is tied (Fig 186). Highly satisfactory results are obtained by this method. The tape is removed after the straining has stopped. Complicated methods such as pudendal neurectomy (Schwesinger 1956) and strengthening of hymenal ring (Brier 1953) have also been reported.

Choice of anaesthesia depends upon the factors (1) whether the foetuses are required to be delivered live (2) condition of the dam and (3) facilities available. Sedation and local infiltration of analgesic will suffice for surgical intervention in the exhausted animals. If the owner does not insist of getting live pups intravenous anaesthesia with barbiturate or inhalation anaesthesia with halothane may be employed.

In appreciably exhausted patients preparative treatment includes intravenous injection of volume expanders such as glucose saline normal saline Ringers solution plasma or whole blood depending upon availability and requirement. Adrenalin may be incorporated if necessary. In cases complicated with septic or putrefactive changes parenteral therapy with suitable antibiotics should be undertaken.

watch for respirations. Artificial respiration may be resorted to, if necessary. The uterine incision is closed with double row of continuous Lembert suture with 3/0 catgut using atraumatic needle.

The abdominal wound is closed with conventional three-tier sutures.

### Caesarean Section

Laparohysterotomy performed usually at the time of parturition for the delivery of the foetus is called caesarian section. This term has come in usage not because Julius Caesar was born in this fashion but because he had proclaimed an order that a woman in advance pregnancy about to die should have this operation. This operation is resorted to when mutation, forced extraction or foetotomy are of no avail and can be undertaken in all the species of domesticated animals.

### Cows

Maternal dystokia due to primary or secondary uterine inertia, incomplete relaxation of cervix, uterine torsion, narrow birth canal, abnormality in pelvis and foetal dystokia due to abnormally large size of foetus, monstrosity and abnormal presentation are some of the indications of laparohysterotomy in cows.

The operation can be performed either in standing position or in lateral or dorsal recumbency. If standing position is preferred, the animal is secured in a trevis or by the side of a wall. In such a position upper right flank is a site of choice. The cow should be quiet and should not be allowed to sit down during operation. Casting and lateral recumbency is generally preferred. After gently casting, the cow is secured by

tying the limbs. The gravid uterus can be approached through laparotomy at any one of the following sites:

- (1) Lower left flank.
- (2) Lower right flank.
- (3) Paramedian.
- (4) Ventrolateral incision above the arcus cruralis.
- (5) Vento lateral oblique incision.
- (6) Ventral mid line.

The choice of site mainly depends upon the surgeon and to some extent upon the facilities available. Deore (1973) preferred the oblique incision on right abdominal wall while as Verma *et al* (1974) have recommended a horizontal incision above the arcus cruralis on right side. These sites are considered better than paramedian incisions.

In most of the cases local infiltration with analgesic solution is considered adequate. Sedative may be required in excited patients.

After preparing the site for surgical operation and local analgesia, long incision about 40 to 60 cm. is taken and skin, fascia, abdominal muscles and peritoneum are cut. If lateral wall is preferred, rather thick muscle bellies of external and internal oblique abdominal muscles have to be cut and on ventral aspects their aponeurotic portion is encountered. Through the laparotomy incision the gravid horn of the uterus is brought as much to the level of abdominal opening as possible. With a separate clean scalpel, the uterus is incised long enough for easy extraction of the calf. Forceful traction of the foetus through uterine opening may result into irregular tare in the wall. The calf is easily removed by grasping the extremities of the limbs which are easily accessible.

If the calf is living the umbilicus is cut and the body surface and nostrils are cleaned. The placenta may or may not be removed through uterine incision. The uterine incision is closed with double row of Lembert sutures using Chromic catgut No 1 and atraumatic needle. The suture line is dusted with crystalline penicillin powder and the uterus is put back in position. The surgical wound on lateral abdominal wall is closed with figure of 8 type of sutures incorporating peritoneum muscles and skin in the same bite. The ventral abdominal incision is closed in 3 layers. The skin is closed by mattress sutures.

Recovery rate varies from 62.5 to 89 per cent. The important factors governing the prognosis include the period lapsed between labour pains and time of operation, exhaustion, general health, toxæmia or septicaemia and degree of shock. Surgical risk in such cases could be considerably reduced by preoperative treatment with volume expanders such as normal saline, glucose saline, Ringers solution, plasma or whole blood depending upon the requirement and availability.

Post operative treatment includes daily dressing of the wound and parental administration of antibiotics.

### Ewes and Does

Indications for caesarian sections in these animals are Ring womb condition, malpresentation, uterine torsion besides those mentioned for cow.

Left paramedian site is suitable in these species. If the approach through the flank is selected, inverted L shaped field block produces satisfactory anaesthesia (Sharma *et al*, 1973). Preoperative care, surgical technique and postoperative management is the same as in cows.

### Hysterectomy, Panhysterectomy or Spaying

Grossly infected uterus, gangrenous and emphysematous puppies, pyometra (cystic hyperplasia of endometrium), diseases of ovaries are the principal indications for surgically removing the uterus, fallopian tubes, ovaries and associated structure like broad ligament of the uterus. Such an operation is called panhysterectomy or spaying. It is also undertaken to prevent oestrus and associated problems such as bloody discharge, attraction of male dogs during oestrous period and accidental mating. Endocrine imbalance resulting in infertility, pseudocyesis (false pregnancy) and mammary tumours also warrant this operation in bitches.

Panhysterectomy can be performed at any age but the age between 4-6 months is considered to be better. As far as possible it may not be done during oestrus and pregnancy. In older animals it may be planned 3 months after last oestrus or 6-8 weeks after whelping.

The animal should be clinically examined and prepared for operation. Withholding food 12 hours prior to operation and an enema before premedication are recommended. General anaesthesia with premedication is best done with the combination of chlorpromazine and short acting barbiturates or morphine and inhalation anaesthesia with ether or halothane. A midline incision starting from umbilicus and extending 3 to 5 cm caudally is taken to cut the skin, fascia, linea alba, falciform ligament and parietal peritoneum. Through the opening, tenaculum or index finger is passed along the inside of abdominal wall below the abdominal viscera and uterus or its broad ligament is hooked. This structure is followed to the opening and the

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The animal should be clinically examined and prepared for operation. Withholding food 12 hours prior to operation and an enema before premedication are recommended. General anaesthesia with premedication is best done with the combination of chlorpromazine and short acting barbiturates or morphine and inhalation anaesthesia with ether or halothane. A mid-line incision starting from umbilicus and extending 3 to 5 cm. caudally is taken to cut the skin, fascia, linea alba, falciform ligament and parietal peritoneum. Through the opening, tenaculum or index finger is passed along the inside of abdominal wall below the abdominal viscera and uterus or its broad ligament is hooked. This structure is followed to the opening and the



left ovary is withdrawn. Suspensory ligament of the ovary may be ruptured with gentle pressure, if necessary. Overstretching may rupture ovarian vessels and cause considerable haemorrhage. Two hemostats are applied over ovarian bursa which is then opened and the ovary is clipped off with curved scissors. The ovarian pedicle is ligated with double ligature of chromic catgut No. 0. The stump is then severed. A careful check on haemorrhage is very important before returning it to abdomen. The same procedure is repeated on the other side. Broad ligament is either torn by digital pressure or cut with scissors avoiding the blood vessels.

The body of the uterus is then brought out of incision. The uterine blood vessels which are on either side are ligated with separate sutures and a third suture is taken to cover the entire cervix. The uterus is cut just cranial to the ligatures. Haemorrhage should be carefully checked. The uterine stump is then sutured with 3/0 chromic catgut using Cushing suture. In some instances, a piece of broad ligament is used to seal the uterine stump.

The abdominal wound is then closed. Obesity and vulvitis occur in many cases since the ovarian hormones are no more available. In a few cases urinary incontinence may also be observed.

### Torsion of Uterus

Torsion of the uterus is the twisting of uterus along its long axis. This condition is frequently encountered in cows and buffaloes and occasionally in ewes and goats. In dogs and cats only one horn is involved in torsion. In mares, torsion of uterus is uncommon as the uterus is dorsally attached to the broad ligament of uterus (Roberts, 1971).

Generally rolling of the dam, rotation of the foetus, induction of vigorous foetal movements, swinging of uterus and intra-abdominal manipulation are some of the methods used in majority of cases for the correction of the torsion of uterus. (These methods have been already described in the chapter on Maternal dystokia).

Before the surgical measures are adopted, the rectal and vaginal examination should always be done to differentiate the conditions such as traumatic recticulitis, pyelo-nephritis or intussusception of bowel which simulate the symptoms of torsion.

In complicated cases of torsio uteri with adhesions, the calf is removed by hysterotomy, the uterus is sutured and then the torsion is corrected. In small animals, complications may ensue if the condition is not diagnosed early.

formed in correct manner, the most commonly encountered complications are: (i) haemorrhage (ii) evisceration (iii) oedema and (iv) infection.

Improper crushing of spermatic artery, due to faulty instrument or technique, leads to haemorrhage. The emasculator must be kept in position for 5 minutes and released slowly. If haemorrhage from the artery is seen while the emasculator is being released the emasculator should be clamped immediately. In delayed haemorrhage the artery is identified and ligated with catgut No. 2.

Faulty closure of the inguinal canal results in evisceration which may occur during first 12 to 18 hours after castration. An immediate detection avoids further serious complications. When evisceration does occur the horse is anaesthetised and cast on dorsal recumbency. In case there is prolapse of bowels in the inguinal canal the mass is gently pushed back to abdominal cavity and the inguinal canal is closed.

Inadequate exercise and drainage from scrotal incision may cause oedema which, if contaminated, may result into septic conditions. Judicious exercise, warm water spray or fomentation and topical antiseptic wound dressing give good results. In the event of contamination, systemic antibiotic treatment is warranted.

### **Bulls, Rams and Bucks**

Castration accompanied with haemorrhage, as is done in horse, is seldom practised in bulls or rams. Crushing the spermatic cord with Burdizzo clamp is adequate to effect atrophy of the testicles. This is possible mainly because of the long scrotal neck. In this method the animal is cast and secured in

lateral recumbency, the testicle along with the scrotum is pulled and the clamp is applied while the spermatic cord is held between the index finger and the thumb otherwise it may slip from the clamp when it is being pressed.

Placement of the clamp over epididymis or testis may result in severe orchitis. A highly painful swelling is seen within 24 hours. Such a complication is treated with cold or hot application depending on the conditions and systemic antibiotic therapy to prevent sepsis.

### **Dog**

The dog should be anaesthetised with short acting barbiturate. An incision on the scrotum is taken to cut the skin, dartos and the tunica vaginalis. The testis along with the cord and the external cremaster muscle is pulled. The cord is separated from the muscle and a surgical knot is tied with the cord and the muscle. No ligation is necessary. This technique has been reported to give good results. The other testicle is removed through the same scrotal opening by incising the median septum.

An alternate site for incision is just above the internal inguinal ring, leaving the scrotum intact. Even though this approach safeguards against evisceration, accumulation of oedematous fluid in scrotal cavity is commonly seen.

### **Cat**

A male (tom) cat can be castrated under local analgesia. Hair on the scrotum are removed by plucking. The testes are exposed by incising scrotum and are pulled hard so that spermatic artery ruptures due to traction and closes by recoiling in young cats. In

older cats the cord is ligated and severed by twisting with two artery forceps placed behind the ligature. The wound is dressed with antibiotic powder to prevent sepsis.

### Pigs

Early castration in swine between one to several weeks is advisable for promoting the growth. In younger lot, the castration is performed under local analgesia. The animal is cast and secured in dorsal recumbency. An incision is taken from median line about middle of the scrotum and extended about equal distance below the bottom of scrotum. The attachments of tunic with the scrotum are separated by passing the finger around and by traction. The testicles enclosed in the tunics are brought out of the incision. The tunics and cord are divided at the anterior end of the scrotum. The other testicle is reached by incising the median septum. Antibiotic powder is dusted on the incision and inside the scrotal cavity.

General anaesthesia — either inhalation or intravenous, is necessary for castration of large boars. The operative technique is similar except that a separate incision is taken for each testicle and that the spermatic cord alongwith tunic is ligated and then divided below the ligature.

### Partial Castration

By adopting this method the passage for the sperms is completely blocked so that the operated animal is unable to reproduce but the production of male sex hormones is not affected. Bulls, boars, rams and bucks when partially castrated are reported to grow faster without much fat deposition. They can also be used as teaser animals.

Methods of partial castration are: (1) Vasectomy, (2) Epididymectomy (German method), (3) Injection of sclerosing agent in epididymis, (4) Baurtjejan's method (Russian method), and (5) Lateral deviation of the penis.

### Vasectomy

The animal is cast and restrained in lateral recumbency. Special method of casting for castration provides convenience for work. Local analgesic is infiltrated at the site of incision. According to the technic of Tharp (1955), anterior aspect of the neck is preferred as external cremaster muscle which is massive in adult bulls covers the tunic to the neck of the scrotum. Fry *et al* (1965) favour the lateral approach for the sake of convenience in holding the testes during operative procedure.

If anterior approach is selected the testis alongwith the scrotum is pulled downward and backward by an assistant. The vas deferens is pressed to the skin and palpated posterior or medial to the spermatic cord and is enclosed in a special fold of tunica vaginalis propria (Sisson and Grossman, 1963). After the skin incision, the vas deferens is identified and separated from the other structures of the cord by blunt dissection. It is then held in two artery forceps set about 2.5 cm. apart and is ligated with No. (0) catgut just above and below the forceps and a piece about 2 cm. long, is cut off. The skin is closed with a cruciate suture or by 2 or 3 simple interrupted sutures using synthetic fibre.

Vasectomized bull should not be allowed to move with cows for at least 3 weeks. According to Frank (1959), the bulls to be vasectomized should be young and should not have been used

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Vasectomized bull should not be allowed to move with cows for at least 3 weeks. According to Frank (1939), the bulls to be vasectomized should be young and should not have been used

previously for breeding purpose. However, a great caution and concentration is required for correct identification of the vas deferens which is often indistinguishable even in adult bulls (Fry *et al*, 1965).

### Epididymectomy (Caudectomy)

A simpler, quicker and equally effective method is devised by Rosenberger (1956). In India Hattangady and George (1968) successfully tried this method for making teaser bulls. The base of the scrotum is anaesthetised by injecting 3 to 5 ml. of 2 per cent lignocaine or procaine hydrochloride. The testicle is pressed downward and an incision of about 2 cm. is taken over the bulge; the cauda epididymis is exposed and is grasped in artery forceps (Fig. 187). A curved needle with double catgut thread is passed between cauda and the testis. The thread is cut near the needle so that two separate threads are available. They are tied at two places at a distance of 1.5 cm. from each other. The piece of cauda epididymis is cut with a curved scissors. The scrotal skin is closed with two inter-

rupted sutures or may be allowed to heal by granulation.

### Use of Sclerosing Agent

Dorn (1956) reported that effective partial castration could be performed by injecting 2-3 ml. of 'Dondren' (a sclerosing agent by Knoll Co., Germany) into the lumen of cauda epididymis of a bull. Histopathologic examination of the cauda epididymis at different intervals after the injection shows local inflammation followed by granulation and total obstruction of epididymal canal. The testicles are pressed downward against the scrotum. The cauda is palpated and a fine bore needle is introduced into the lumen of epididymis. The drug is injected while the needle is withdrawn so as to deposit the drug at different spots. Dorn (1966) has further reported that the method is safe and effective. However, this method has not yet been widely accepted perhaps due to the fear of recanalization.

### Balburtejan's Method (Russian Method)

Based on the observations made during the second World War, that the horses with intact epididymis and a small portion of testicular tissue behave as entire horses, Balburtejan (1963) introduced a new method of castration of farm animals. After casting and securing the animal the area is prepared for surgical intervention. The testis is held in the left hand, pressed firmly against the lateral wall of the scrotum. A stab incision of 1.5 to 2 cm. is made in the middle third of the scrotum (Fig. 188). The scalpel is rotated through 120° to 135° so as to facilitate pressing

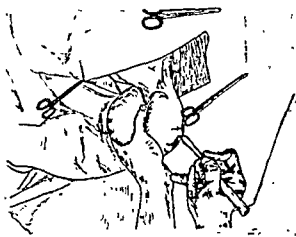


Fig. 187, Caudectomy — Incision on the bulge.



Fig 188 Caudectomy — A stab incision on the scrotum

out of parenchyma. With the bent fingers of right hand the testicular parenchyma is pressed out (Fig 189). If necessary a scoop may be used. About two thirds of the parenchyma should be removed.

As the testicular parenchyma becomes denser with the advancement in age a great difficulty is experienced while removing it in calves above 4 months in age. Hence it is recommended that this method should be undertaken in the animals below the age of 4 months.

*Lateral deviation of penis* and or post scrotal penile amputation have been suggested for making teaser bulls (Straub and Kendrick 1965, Deshpande and Kulkarni 1969). Kulkarni (1973) found epididymectomy to be simpler and easier than this method.

### Prepuce and Penis

Traumatic injuries such as laceration and contusion are generally encountered during breeding season especially during the act of coitus or soon thereafter.

Laceration to internal prepuce leads to inability to protrude penis and is characterized by a swelling of the prepuce. Since the preputial cavity usually harbours ubiquitous organisms such

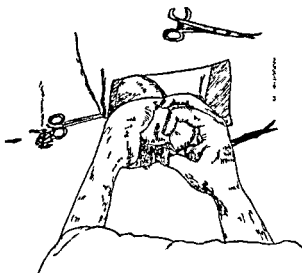


Fig 189 Caudectomy — Baiburtchjan's method

as *Pseudomonas corynebacterium*, *Staphylococcus* or *Streptococcus*, the lacerated wounds should not be sutured. Attempts should be made to prevent necrosis of the elastic tunic of the prepuce. In cases of fresh lacerations the prepuce is flushed with mild non-irritant antiseptic solution followed by antibiotic in propylene glycol base. This treatment may have to be continued for a week or so. Systemic antibiotic therapy may be resorted to if considered necessary. The bull should be given sexual rest for a month or so till the penis can normally be extended. This can be checked by manual extension. Any premature action of putting the bull to service will damage the newly healed tissue. A purse string suture is necessary in some cases to prevent prolapse of the prepuce.

If the haematoma or oedema develops the prepuce may get locked or remain in prolapsed condition. With the normal pull of retractor muscle of penis the lacerated wound is widened and presents a transverse appearance. In such cases conditioning of the tissue to be operated is very necessary. This is accomplished by spraying the area daily with warm water for half an hour to

remove foreign material deposited on the prepuce and flushing away the necrotic tissue. The area is then smeared with the following mixture:

Lanolin ...	450 gm.
Scarlet ...	60 gm.
Tetracycline ...	2 gm.

After this daily treatment till granulation covers the area, a minor surgery is undertaken. Under local analgesia the granulation tissue and fibrotic tissue that may be encountered are clipped off with a pair of curved scissors until neat fresh mucous membrane is seen. The prepuce is returned to normal position and purse string suture is taken over the preputial opening.

Cases complicated with abscessation are generally considered to have unfavourable prognosis. The abscess usually becomes apparent 3 weeks after the preputial laceration. A spherical, semi-fluxing mass is palpable in preputial cavity. Surgical excision of the abscess from an external preputial approach invariably fails as new abscess develops postoperatively. If the abscess is small enough to allow extension of penis, the abscess alongwith the pieces of mucous membrane of the internal prepuce is removed but if the abscess is large enough to obstruct extension of penis it is advisable to wait till the infection subsides. The fibrotic tissue is then removed surgically.

### Prolapse of Prepuce

In heavy breeds with pendulous sheath, such as Sahiwal, Red Sindhi, Deoni, Gir, Kankrej and others, preputial prolapse with fibrosis is occasionally seen. Minor lacerations and trauma is considered to be the primary cause. A mass of preputial mucous membrane with fibrosis is seen hanging out of the preputial orifice.

Conservative treatment is of no avail. The prolapsed mass has to be surgically removed.

### Operative Technique

After overnight fasting, 30 gm. of chloralhydrate should be administered as a drench to the bull an hour prior to the operation. Trimeprazine tartrate (Vallergan, M & B.) 500 mg. may be administered intravenously. Then the animal is cast and controlled in a position of lateral recumbency. The preputial hairs should be clipped and lignocaine hydrochloride 2 per cent solution is used as local infiltration anaesthesia. Operative technique devised by Hattangady *et al* (1968) is recommended.

The prolapsed mass is pulled anteriorly by applying 4 Allis forceps so as to expose the healthy tissue. Four to eight suture threads (Vetafil) are passed through the exposed healthy portion by using a straight needle in a criss-cross fashion well above the prolapsed mass and are held by an assistant. This is done to prevent the layers from being retracted into the prepuce after the excision of the prolapsed mass. Then a tourniquet is applied above the strands over the prepuce taking care to exclude the penis. A circular incision is made in the healthy tissues in the external layer above and around the base of the prolapsed mass. Care should be taken to retain the preputial orifice intact and to conserve as much healthy tissue as possible. The dissection is continued into the deeper layers in an upwards or a downward slanting fashion depending upon the extent of prolapse until the internal layer is reached. A similar incision is then made in the internal layer anterior to the previous inci-



sion in the external layer thus removing completely the prolapsed mass. After checking haemorrhage, the cut edges are co-opted by inserting continuous subcuticular sutures using No 0 catgut. A smaller bite of lesser circumference is taken in the internal layer so that co-optation of the layers after suturing is in perfect alignment. A few interrupted sutures are inserted for reinforcement. Finally when the strands are pulled out and the tourniquet released, the sutured portion recedes into the sheath.

Parenteral administration of antibiotics for one week is recommended. The wound is dressed daily using sulphamylamide or penicillin in oily base.

Post operative stricture of the preputial orifice is one of the complications (Frank, 1939; Lenert, 1956). If every thing proceeds normally the bull can return to service in about 2 months.

Pendulous sheath has hereditary predisposition as such surgical intervention has limited scope, since such bulls propagate the very undesirable character in their progeny.

Contusion of prepuce or penis when the latter is in extended position, erection of penis through stenotic ring, paralysis of penis due to spinal diseases or trauma, rabies, dourine (in horses only) are the important aetiological factors. A few cases are reported after castration or use of tranquillizers.

Prognosis is not much favourable. It largely depends upon the degree of trauma and promptness of treatment.

Cold packs and supporting bandage may help in reducing swelling and subsequent trauma to penis.

In the cases due to cicatrization of preputial orifice, the orifice is enlarged by surgical intervention and after putting the penis into the cavity a purse string suture is put over the opening to prevent recurrence. In case, paraphimosis is accompanied with necrosis the affected portion of penis is amputated.

This condition is occasionally encountered in bulls. Removal of necrotic tissue and dressing with antibiotics in oily base is found beneficial.

accidentally slips over the glans during a powerful thrust causing balanoposthitis.

Treatment includes sexual rest and douching the prepuce with acriflavin solution, oily preparations such as 1 gm. iodine crystals in 5 litres of mineral oil, or 25 gm. Bismuth formic iodine in 500 ml. of mineral oil are also recommended. Antibiotic ointments are applied daily. The penis is gently taken out at regular intervals to avoid adhesions. Tranquillizers or pudendal nerve block obviate the pain.

### Pudendal Nerve Block in Affections of Penis

In the bovine it is often found difficult to withdraw the penis from the prepuce due to the well developed retractor penis muscle. Complete relaxation and anaesthesia of the bovine penis is necessary in conditions such as haematoma of the penis, catheterization, fractured penis, injuries and lacerations, removal of warts, tumours, dressing and curetting of the wounds. In addition to this, for the proper examination of penis in conditions such as inability to normally protrude the penis, deviations of the penis, phimosis and in venereal diseases such as, Trichomoniasis and Vibriosis, it is essential to have pudendal nerve block for achieving full relaxation for the ease with which the organ can be exposed for the treatment. Bhokare (1975) in his experimental trials on entire group of animals for causing relaxation of penis achieved success in 64.2 per cent by the method of pudic nerve block.

The technique of pudic nerve block, though a little cumbersome to adopt, is definitely superior to epidural anaesthesia as it gives more consistent results and

the hazards of casting and recumbency for the duration of a prolonged period can very well be avoided.

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# Chapter 58

## Reproduction in the Camel

Camel has its importance in the desert areas of the world as a means of transport and for production of milk, meat and skin. It is possible to use them in arid regions due to their ability to thrive on the fibrous vegetation available over there, which cannot be utilised by any other species of domestic animals. They have a commendable power of endurance in prolonged drought and their capacity to live without drinking water for prolonged periods is admirable.

There are about 9 million camels in the world, 2/3rd of which are located in the tropics and 1/3rd of these are located in the east of tropical Africa. In the modern days of mechanization their number is on the decrease. However, they still maintain their usefulness as meat producers and means of transport since no other species can thrive in arid region.

The camel is one of the oldest mammals now living and probably one of the earliest ever domesticated to serve the mankind in the form of transport, milk, meat, hide, agricultural operations and its fleece utilised for tents, ropes, clothes etc.

In Kenya and Somali Republic camels are chiefly maintained for meat and milk

production. There are two types of camels namely, *Camelus dromedarius* or the Arabian Camel having a single hump and found in the tropics and *Camelus bactrianus* which is double humped and found in the intense cold regions. The bactrian camel is commonly found in Central Asia, Russia, Mangolia and Turkey while the Arabian camel is found in Southern Asia and Northern and Eastern Africa. In the single humped camel there are two types (i) Riding camel and (ii) Baggager. Although there are a number of breeds within these types, the differential features cannot be markedly distinguished as in cattle. The camel of each tract however carry hereditary traits which make them distinct from one another. Family lines also exert a distinguishable stamp of their features. Environmental conditions have a marked influence in building each type for instance, there is much more in common between the Egyptian delta camel and the camel found in Sind (Pakistan) developed under riverine conditions, than between the Sindhi and Kuchia developed in the hills. The hilly types of Kuchi camel is compact, muscular and heavy boned reaching a height of only 1.8 to 2.0 meters at withers while the Egyptian and Sindhi may reach upto 2.14 meters. In

the plain camels the conformation and performance vary to a great degree between the light boned, fine boned, thick skinned, alert, desert riding type and the massive baggage type from the riverine areas which are used to good living and regulated work.

Camel in India are medium in size, strongly built and having active habits. They are famous for stamina, endurance and are therefore, extensively used for riding purposes in the desert. The average Indian camel weighs 531.7 to 557.9 Kg. (Leese, 1927). In a well bred healthy camel, the limbs should be well set and straight. The feet should not be pointed outwards, the elbows should be cramped inwards and the hock should not show a tendency to touch each other while at rest or on march. The conformation should ensure a free easy straight gait which is essential for the work camel. A camel selected for work should be in a plumpy condition with a rounded hump and well sprung ribs with plenty of muscles on the loins. It should be able to sit and rise with ease when loaded.

Bikaneri camel is an essentially riding camel and may be regarded as the parent animal of this area in India. It is found in the desert of Rajasthan i.e. Bikaner, Churu and Sriganganagar from where they migrated to Punjab, Uttar Pradesh, Madhya Pradesh, Gujarath and a few in Mysore and Deccan. Strictly speaking the home of Bikaneri camel is the sand tract of Bikaner division where the average rainfall is 100 to 200 m.m. and temperature as low as 5°C. in the winter and 49°C in summer. The animals are kept in herds by professional breeders locally known as 'Raikas'. A good riding camel can cover very long distances as much as 96 to 112 km. a

day. It can travel 48 km. a day comfortably for a number of days at stretch, the average speed being 10-11 km. an hour.

### Management

At the breeding farms, the practice is to let loose the animals for grazing in the morning and they return in the evening. According to age the animals are divided into different groups. The studs are allowed to move with the nonpregnant group of females during the breeding season from November to March. The newly calved dams and their young ones are kept separate from the herd and are allowed to stay at the farm during the day time for about a month before they are turned out for grazing, while at night they are enclosed separately.

The camel can easily be trained for work. Both males and females serve equal purpose. At two years of age the camel is usually introduced to the discipline of control either by putting a head rope or by fixing a nosepeg made of wood, bone or metal and is passed through the nose below and towards the extremity of nasal bones. As the camel reaches 4 years, it is loaded with empty saddle and by and by accustomed to the gradual increase in the load as well as gets used to the head control and trained to sit and rise at command. The she-camel is usually rested and allowed to graze from the mid-term of pregnancy till such a time that the calves are weaned. The males are usually put to work at about 4 years and given light load. During the next 2 years light pack-work is taken and full load is only given when they are 6 years old.

Castrated males serve better purpose as their work is not interrupted due to

# Chapter 58

## Reproduction in the Camel

Camel has its importance in the desert areas of the world as a means of transport and for production of milk, meat and skin. It is possible to use them in arid regions due to their ability to thrive on the fibrous vegetation available over there, which cannot be utilised by any other species of domestic animals. They have a commendable power of endurance in prolonged drought and their capacity to live without drinking water for prolonged periods is admirable.

There are about 9 million camels in the world, 2/3rd of which are located in the tropics and 1/3rd of these are located in the east of tropical Africa. In the modern days of mechanization their number is on the decrease. However, they still maintain their usefulness as meat producers and means of transport since no other species can thrive in arid region.

The camel is one of the oldest mammals now living and probably one of the earliest ever domesticated to serve the mankind in the form of transport, milk, meat, hide, agricultural operations and its fleece utilised for tents, ropes, clothes etc.

In Kenya and Somali Republic camels are chiefly maintained for meat and milk

production. There are two types of camels namely, *Camelus dromedarius* or the Arabian Camel having a single hump and found in the tropics and *Camelus bactrianus* which is double humped and found in the intense cold regions. The bactrian camel is commonly found in Central Asia, Russia, Mangolia and Turkey while the Arabian camel is found in Southern Asia and Northern and Eastern Africa. In the single humped camel there are two types (i) Riding camel and (ii) Baggager. Although there are a number of breeds within these types, the differential features cannot be markedly distinguished as in cattle. The camel of each tract however carry hereditary traits which make them distinct from one another. Family lines also exert a distinguishable stamp of their features. Environmental conditions have a marked influence in building each type for instance, there is much more in common between the Egyptian delta camel and the camel found in Sind (Pakistan) developed under riverine conditions, than between the Sindhi and Kuchia developed in the hills. The hilly types of Kuchi camel is compact, muscular and heavy boned reaching a height of only 1.8 to 2.0 meters at withers while the Egyptian and Sindhi may reach upto 2.14 meters. In

the plain camels the conformation and performance vary to a great degree between the light boned fine boned thick skinned, alert desert riding type and the massive baggage type from the riverine areas which are used to good living and regulated work.

Camel in India are medium in size strongly built and having active habits. They are famous for stamina endurance and are therefore extensively used for riding purposes in the desert. The average Indian camel weighs 531.7 to 557.9 Kg (Leese 1927). In a well bred healthy camel the limbs should be well set and straight. The feet should not be pointed outwards the elbows should be cramped inwards and the hock should not show a tendency to touch each other while at rest or on march. The conformation should ensure a free easy straight gait which is essential for the work camel. A camel selected for work should be in a plump condition with a rounded hump and well sprung ribs with plenty of muscles on the loins. It should be able to sit and rise with ease when loaded.

day. It can travel 18 km a day comfortably for a number of days at stretch the average speed being 10-11 km an hour.

### Management

At the breeding farms the practice is to let loose the animals for grazing in the morning and they return in the evening. According to age the animals are divided into different groups. The studs are allowed to move with the nonpregnant group of females during the breeding season from November to March. The newly calved dams and their young ones are kept separate from the herd and are allowed to stay at the farm during the day time for about a month before they are turned out for grazing while at night they are enclosed separately.

rut period nor their attention is distracted by females. It is necessary to castrate them only after they have attained full size which is usually reached between 4 to 6 years of age.

### Feeding

In the natural course the camels usually prefer to browse rather than to graze upon. They take long time even as that of 4 to 6 hours for consuming or foraging their food and another 4 to 6 hours for ruminating. They do not like to forage during intense heat of the day. In certain territories camels live entirely by browsing on low bush plants such as camel thorn (*Alhagimaurorum*) and salt worts such as *Haloxylon recurvum*. Some camels do not like the grass when it is green. In contrast to this in Somali Republic, the grass forms the principal portion of the camel's food. Camels can be stalled and kept in good

condition like cattle on conserved fodder and grains. Stallfeeding is however very expensive. A certain amount of hand feeding is usually done to supplement browsing or grazing. Gram legume straw, millet, chaffed wheat straw, jowar, maize, wheat bran and salt are fed in adequate quantities supplementary to 10 kg. or more of hay. Mathur (1960) reported the following scale of ration for camel: barley crushed 2.5 lbs, gram crushed 2.25 lbs., green fodder 4 lbs., bhusa 16 lb., rock salt 5-oz., common salt 6.2 oz. The other well known camel feeds are: Moth chara (*Phaseolus aconitifolius* straw), Beri pala (*Ziziphus jujuba*), Khejri leaves (*Prosopis spicigera*), Acacia leaves (*Acacia arabica*), Phok (*Calligonum polygonodis*), and Seven grass chopped (*Elionerus hirsutus*).

The schedule of ration scale at Government camel breeding farm, Bikaner (Rajasthan) is as follows :

Particulars of the animals	Fodder (lbs)	Concentrate (lbs)	Salt (Ozs)
1. Under one year	4	1	1
2. 1 to 2 years age	8	2	2
3. Animals of 3 years	12	3	3
4. Above 3 years age	16	4	4
5. Stud camels	18	5	5

There is seasonal variation in the above ration schedule depending on the forage conditions of the pasture. Extra ration is provided to animals in advance pregnancy, soon after parturition and to stud camels during the breeding season.

### Watering

In the camel, loss of water through evaporation from the body surface is much less on account of the wooly coat

which insulates the body from surrounding heat. It can withstand dehydration to an exceptional degree. They can even afford to stand to reduction of 40% of its body water. Such a low degree would prove fatal to any other species. It is believed that the camel drinks only enough to compensate for the amount of water lost from its body and there is no store for emergency. The quantity of water consumed may extend anything



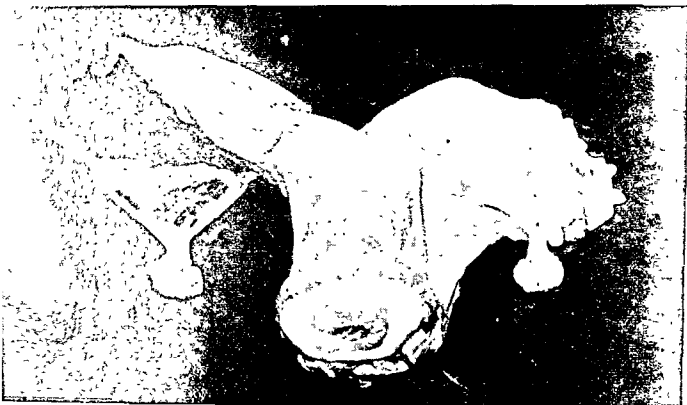


Fig. 193. Uterus and ovary of a she camel.

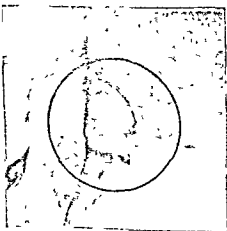


Fig 194. Udder of a she camel.

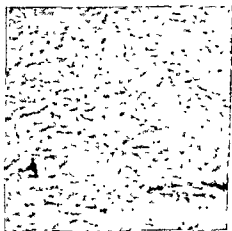


Fig. 195 (a) Showing typical fern pattern (arborization) during oestrus.



Fig. 195 (b) Showing a typical pattern after end of calf heat.



Fig 195 (c) Showing 'nil' pattern after end of calf heat.



from 50 to 100 litres at a time depending on the interval between watering. If possible camel may be watered every day and if done so they might consume 30 to 36 litres. If they have been deprived for considerable number of days they may consume even upto 90 litres at one time. Indian desert camel needs water every second day others which are on prolonged march may withstand for 4 days. In Somali Republic the camel have exceptional endurance in this respect and they can live without water for days together.

## ANATOMY OF REPRODUCTIVE ORGANS

### Male

#### TESTES

The testicles are located high up in the perineum behind the thighs (Fig 190) a few cm below the ischial arch in the same position as that in the dog. The posterior border is slightly higher than the anterior (Fig 191). The attached border is concave and dorsal and the free border is convex and ventral. Compared to the body size of the camel the testicles are small. The testes in camel are oval and in some these are descended into the scrotum at birth and are situated in perineal region in adult life. In some camels the testicles are not descended into the scrotum until about the age of six months. The left testis is slightly heavier than right one in adult camels.

The capsule of the testis is composed of irregularly arranged dense fibroblastic tissue. The elastic fibres play an important role in seasonally breeding animals in which the testis become turgid during rutting period and flaccid during the non rutting period. The interstitial

tissue is rich in polyhedral cells of Leydig's. The amount of interstitial tissue varies with the periods of the year maximum in rutting periods and reduces during the non rutting periods.

The spermatozoa are seen in the lumen of tubules in adult animals of all ages during all periods of year. Spermatozoa appear pointed and spear shaped in profile and oval when observed in flat surface. It appears that spermatogenesis is a continuous process in camels throughout the adult life irrespective of rutting and non rutting periods (Singh 1970).

#### Epididymis

The epididymis is relatively larger than testis in prenatal life and in the dry old calf. In young the epididymis covers almost the entire anterior surface of the testis. In adult animals it runs obliquely on the anterior surface of the testis. The weight of the epididymis ranged from 10 to 10 gm in adult animals. The lumen varies from 15 to 300  $\mu$  diameter in different parts of the ducts of adult camels during all the periods of the year. lumen contains spermatozoa. The mucosal folds and thickness of the muscular layer progressively increases towards the tail region.

#### Excurrent ducts and accessory sex glands

The epididymis is attached to the attached border. It is covered by the tunica vaginalis and surrounded by the albuginea (Gray 1971).

The interstitial tissue of the testis to admit only one spermatozoa into the lumen.

The interstitial tissue of the testis is the same.

The urethra in its intrapelvic portion is big enough in caliber to admit a pencil. However the extra pelvic portion progressively becomes smaller in caliber so that when it enters into the penis it may not admit even a dog catheter. At the ischial arch the urethra forms posteriorly a cul-de-sac about 2.5 cm. deep. In camel, the penis and the urethra have two successive curves i.e. sigmoid flexure as in the bull, the first has its convexity forwards and the second has its convexity backwards. The only difference is that in bulls the sigmoid flexure is post-scrotal whereas in the camel it is pre-scrotal. During erection the penis is straightened out to a total length of about 68 cm. from the ischial arch. Retraction of the penis is effected by the action of muscles inserted into the second curvature. The penis gradually tapers to its tip which however is not pointed. The urethra is located in a groove below the corpus cavernosum. The tip of the penis has a very weird arrangement. About 3 cm. from the end of the tip, the portion is not flexible as well as not symmetrical. The extremity is formed by a curved cartilagenous expansion with a round edge. Its concavity is inwards. At the base of this concavity, there is a slender, pointed and inflexible process about 0.5 cm. in length and out of this process an extremely small orifice of the urethra opens at about 2/3rd of its length. At the base of one side of this little process there is a small loose fold of mucous membrane. The urethral orifice is too small to admit even a knitting needle.

The penis is directed forwards and not backwards and its tip is normally about 10 cm. from the opening of the prepuce or sheath.

The sheath or prepuce is sufficiently large, flat, pendulous and triangular in

shape. The orifice at the free angle is turned backwards so that when the camel stales, the stream of urine is directed behind him. A finger or a thumb can be admitted in the orifice of the sheath. The sheath has powerful muscles for extending it forwards or pulling it backwards. The skin over the sheath is black, covered with short hair and bears two rudimentary nipples on its side.

### Camel Spermatozoa

In domestic animals spermatogenesis is a continuous process and the adult male is usually ready for coitus at any time of the year with the exception of certain species like deer, camel and elephant which show their sexual activity in a restricted period of the year in a definite season of rut and spermatogenesis occurs during this period only. So far little work is done on camel semen. Micrometry of 2100 spermatozoa from 21 camels revealed the average head length, head width, mid piece length, tail length and the total length of the spermatozoa as 5.800, 3.030, 7.318, 35.933 and 49.054 microns respectively (Madan, 1969). The head shape is more elliptical rather than ovoid while the head width being widest at equatorial segment and then narrows towards the anterior end. In general the camel spermatozoa are smaller than those of other species of domestic animals (Fig. 192, a, b, c, d). Similar findings were reported by Raoup and Naggar (1965) on Egyptian camel.

The mean percentage of the anterior and posterior protoplasmic droplets and spermatozoa without droplets in the head region and epididymis were 63.19, 6.28 and 29.73, in the body region were 21.56, 39.02 and 39.42 and in all re-

gions were 8.88, 36.42 and 54.70 respectively. The work on spermatozoa in different parts of epididymis clearly indicates that as the sperms move from caput epididymis to cauda epididymis the droplets move distally showing the maturation process as in other species.

## Female

### OVARY

The gonads of the female camel are somewhat reddish flattened lobulated with more or less circular outline the medial and lateral surfaces being slightly convex (Shalash 1965). In a sexually mature female the organ appears like a bunch of grapes due to the presence of numerous ovisacs on the surface of the ovary. In pregnant animals the shape varies considerably due to presence of corpora lutea of various sizes at different positions.

The camel ovaries are about 4.5 cm long and 1.5 cm thick weighing about 15 to 20 grams (Grossman 1960). The findings of Shalash (1965) are presented in Table 81.

The ovaries are situated in the sub-lumbar region under the 6th or 7th vertebra. According to Shalash (1965) the ovary is enclosed in a large conical pocket like fold of mesovarium known as the ovarian bursa the apex of which is blind and laterally situated whereas

the medial end forms a large circular orifice beyond which laterally the fimbrial end of the uterine tube is situated on the floor of this bursa. The ovarian ligament a cord like structure is found to extend from superior surface of broad ligament to the hilus of the ovary of its own side. According to Tayeb (1948) there is no ovulation on the camel ovaries and there is attachment between the fimbriae and the ovary.

### GRAAFIAN FOLLICLES

Numerous Graafian follicles are found in various stages of development on the ovaries as in other species. They are usually situated on the free ventral borders at the anterior pole on the lateral surface at the posterior pole and on the medial surface of the ovary. They are clear translucent spheres without stigma and the greater part of the bulk protrudes over the surface of the ovary. The peculiarity in this species is the presence of Graafian follicles at different developmental phases along with the active corpora lutea of pregnancy. It seems that the presence of a corpus luteum in the ovary does not necessarily prevent a Graafian follicle from being increased in size. Graafian follicles were seen in 182<sup>nd</sup> pregnancies (Shalash 1965). The average diameter of the Graafian follicle is  $1.20 \pm 0.15$  cm for those developed in the left

Table 81  
SIZE AND WEIGHT OF CAMEL OVARIES

Type	Diameter/cm		Weight/gm	
	Right	Left	Right	Left
Non functional Ovaries	$1.82 \pm 0.35$	$1.90 \pm 0.32$	$3.65 \pm 1.49$	$3.97 \pm 1.87$
Ovaries with Graafian follicle	$2.09 \pm 0.35$	$2.15 \pm 0.36$	$5.51 \pm 2.19$	$5.67 \pm 2.62$
Ovaries with corpora lutea of pregnancy	$2.28 \pm 0.28$	$2.33 \pm 0.33$	$7.84 \pm 2.50$	$8.51 \pm 2.81$

ovary and  $1.24 \pm 0.42$  cm. for those which occur in the right ovary.

### CORPUS LUTEUM

In camel the corpus luteum is seen during pregnancy only except in rare cases with patent os uteri (Shalash, 1965). During early stage of pregnancy it is soft and flabby in consistency and spherical in form (at times oval) protruding wholly from the surface of the ovary. In later part of the gestation period the thecal layer becomes whitish opaque concealing the colour of the lutein mass so that the intact corpus luteum appears grey, whitish grey or bluish grey. There is increase in connective tissue and decrease in lipoids and fats in lutein cells. About a week after the calving or abortion the consistency of the corpus luteum becomes hard and is compressed laterally. The colour of old corpus luteum is blackish brown. The regressing corpora lutea decrease in dimensions first transverse then longitudinally in an alternate fashion. The degeneration of lutein cells in the regressing corpus luteum is due to the presence of thickened thecal capsule and invasion of the connective tissue surrounding each individual cell and depriving it of its blood supply.

The diameter of right and left corpus luteum of pregnancy is  $1.85 \pm 0.3$  cm. and  $1.88 \pm 0.36$  cm. and the weight being  $4.15 \pm 1.69$  gm. and  $4.68 \pm 2.07$  gm. respectively (Shalash and Nawito, 1964).

Since the corpus luteum in camel is present only in pregnancy it is theorised that probably some stimulus such as copulation, mechanical or electrical stimulation of the cervix or other apparent stimuli are necessary for ovulation. Barmintsef (1951) reported that

ovulation in female camel occurs during the act of copulation under normal conditions.

### Fallopian tubes

The fallopian tubes are long and flexuous. They follow a course over a pouch formed by a folding over the free edge of broad ligament which envelopes the ovary. The oviduct joins the uterine horn with a small orifice opening on the apex of a conical papilla in the anterior end of cornua. The uterotubal junction is comparatively much hard, however, no histological investigation has yet been reported.

### Uterus

The camel uterus is bicornual, and is similar in form to that of mare (Fig. 193). It is chiefly situated in abdominal cavity and partly extending in the pelvic cavity. With the help of two folds of peritoneum, it is attached to the sublumbar region and pelvic cavity. It is smooth and reddish white in colour with the dorsal surface convex and the ventral surface plane. The organ consists of two horns, the body and the neck. The uterus is divided internally in two compartments. There is a longitudinal septum throughout its length. The body of the uterus is very short, but the horns are long. The broad ligament being long, the free portion of the horns turn outward and downward lying completely in abdominal cavity. The two horns of the uterus diverge anteriorly forming a T shape with the body of the uterus (Lesbre, 1903; Leese, 1927; Tayeb, 1948; Barmintsef, 1951; Shalash and Nawito, 1964). The right horn is usually smaller than the left one. The endometrium is smooth and presents no cotyledons.

Shalash (1965) reported the diameter and length of uterine body, length of right and left horn, to be  $2.11 \pm 0.29$ ,  $5.3 \pm 0.90$ ,  $13.42 \pm 1.76$ ,  $17.45 \pm 2.86$ ,  $3.73 \pm 0.63$  and  $4.29 \pm 0.90$  cm during the follicular phase and  $1.85 \pm 0.40$ ,  $5.84 \pm 0.94$ ,  $13.37 \pm 2.15$ ,  $17.53 \pm 3.29$ ,  $3.73 \pm 0.69$  and  $4.99 \pm 0.91$  cm during inactive stage of ovaries respectively.

The body of the uterus is cylindrical, flattened dorsoventrally being partly abdominal and partly pelvic. It is related dorsally to the rectum and ventrally to the other parts of intestine. The neck or the cervix is constricted and joins with the vagina. It consists of 3 to 4 rows of outgrowing ridges. The cervical canal protrudes about 1 cm posteriorly in the vagina forming two blind cavities, one dorsal and one ventral. The size of the cervical ridges varies with the stage of reproduction. The average length and diameter of the cervical canal during the follicular activity is  $5.32 \pm 0.98$  cm and  $5.96 \pm 0.92$  cm respectively, while as during ovarian inactivity the length and the diameter are  $4.96 \pm 1.25$  and  $5.79 \pm 0.99$  cm respectively. The uterus of camel in Somalia Republic has been found to be

devoid of intercornual ligament (Luktuke, 1971).

In camel the placenta is of the diffuse type as in the mare and not cotyledonary though the animal is ruminant. The vagina is about 32 cm long and has a number of loose folds behind the os. The urethral orifice opens on the roof of a short blind diverticulum in the floor of the vulva as in the cow and the vulva is not prominent. There is no demarcation line between vulva and vagina. The urethra is of a small calibre and difficulty is always experienced in passing catheter except the one used in dogs. The clitoris is located in the ventral commissure.

The Gartner's canals and the Bartholin's glands are well developed in camel.

### Pelvimetry in camel

Size of the pelvis in camel has been recorded for 20 pelvis of either sex by Sharma (1964). The conjugate and transverse diameter being 21 cm and 17.8 cm respectively. The pelvic cavity is cylindrical in shape and larger in females than males. The findings are as follows (Table 82).

Table 82  
AVERAGE SIZE OF THE CAMEL PELVIS (cm)

	Pelvic inlet			Pelvic cavity			Pelvic outlet		
	C	T	O	C	T	O	C	T	O
Cow camel	24	17	28	25.5	19	4	23	13	16
Male camel	20	16.5	22.5	23	17	17	25	12	13.73

N. B.—C—conjugate diameter, T—transverse diameter, O—oblique diameter

### Area, Shape, Pelvic index etc

	Area of inlet in Sq. cm.	Shape	Pelvic index	U.S.A. cervix in cm	Large and symmetrical in cm
Cow camel	427.20	1.35	185	24.6	17.6
Male camel	330.00	1.21	121	21.5	15.0

## Pelvic inlet in camel.

	Conjugate	Transverse
Female	21.9 cm.	17.5 cm.
Male	18.4 cm.	15.6 cm.

The pelvic dimensions in the she-camel are further subjected to variation due to incomplete ossification of the pelvic symphysis even in the adult animals.

**Mammary glands**

The udder is placed on either side of median plane in prepubic region and has four quarters with four teats (Fig. 194). The partition between the two lateral halves is much thicker than that dividing the anterior quarters from the posterior ones. The front or the anterior quarters are comparatively larger than the posterior ones. The teats are short and each teat has two orifices. The skin over the udder is blackish. The lymph glands of the udder are located on the outside of posterior quarters.

The mammary vein becomes very prominent during the lactational phase and has tortuous course under the skin of the belly in front of the udder.

**REPRODUCTIVE BEHAVIOUR****Female****SEXUAL MATURITY**

She-camel reaches sexual maturity at about 3 years of age but usually they are not bred until they are 4 years. Generally the first service is allowed at the age of 4 years so that the first calf is delivered when the cow-camel is 5 years. If breeding is done at an early age of 3 years, it checks the growth of the dam and the calf rarely develops

well. Studies at Government camel breeding farm, Bikaner showed the age at first calving as 1479 days. It was also noticed that maximum calving (40.48%) took place in the month of February and minimum in the month of May.

**Oestrous cycle**

The duration of oestrus is on an average for 3 to 4 days (Williamson and Payne, 1964). Oestrous cycle in camel of Rajasthan varies from 16 to 22 days and 19 days on an average (Vyas and Pareek, 1969). Cases have also been recorded with longer intervals between two oestrous periods. If the cow-camel does not conceive upto March the ovaries become quiescent and inactive but again when the temperature becomes low and the humidity rises up i.e. November and December, the she camel starts coming in heat again and may repeat every 19 days till she conceives. The service generally takes place on second day of heat mostly in the early morning hours or late in the evening and less frequently during the day time. Decreased temperature and high humidity favours service.

The mean duration of oestrus is  $5.00 \pm 0.26$  days (Joshi, 1972). During oestrus, female exhibits typical symptoms of restlessness, bleating and grunting, frequent micturition and typical courtship behaviour. Female in heat accepts male by immediately sitting and adopting a mating stance. The copulation lasts on an average  $24.13 \pm 0.40$  minutes ranging from 21.16 to 27.10 minutes. Other clinical symptoms are swelling of vulva, gradual separation of vulval lips with exposure of part of vagina, slight mucus discharge, vaginal m.m. pink in colour and moist. The vaginal epithelium of females in

oestrus show presence of superficial cells; anestrus stage show presence of basal/parabasal cells and in pregnant animals intermediate type of cells are found.

### Temperature during oestrous cycle

Vaginal temperature during oestrus is  $99.32 \pm 0.09^{\circ}\text{F}$  and  $98.56 \pm 0.103^{\circ}\text{F}$  in dioestrus. Rectal temperature during oestrus is  $98.57 \pm 0.098^{\circ}\text{F}$  and  $97.89 \pm 0.083^{\circ}\text{F}$  during dioestrus. Both vaginal and rectal temperatures show sharp decline on the last day of oestrus.

Pulse rate during oestrus is  $45.18 \pm 0.18$  and in dioestrus  $35.61 \pm 0.35$  per minute. Respiration rate is  $7.22 \pm 0.13$  and  $6.95 \pm 0.11$  respectively.

### pH:

pH of mucus during oestrus is  $7.5 \pm 0.20$ ; in dioestrus  $6.77 \pm 0.26$  and during pregnancy  $7.50 \pm 0.16$ .

### Sodium Ion Concentration of the mucus

Sodium ion concentration of mucus is  $194.10 \pm 14.13$  in oestrus,  $156.46 \pm 19.80$  in dioestrus and  $174.40 \pm 17.61$  milliequi/lit. in pregnant animals.

### Fern pattern

At initiation of oestrus, vaginal mucus shows a typical fern pattern which gradually becomes atypical towards the end of heat. This pattern altogether disappears during dioestrus stage (Fig. 195 a, b, c).

### Calf heat

The appearance of calf heat following parturition is 8 days and lasts for an average 23.59 hours. The oestrous discharge shows the presence of blood cells and other lochia cells.

### Inter-oestrous length

The length of oestrous cycle is  $23.40 \pm 0.22$  days with a range of 22 to 24 days. The pattern of oestrous cycle in camel was found similar to that in equines, with long duration of oestrus and comparative short dioestrus period.

### Signs of oestrus

Oestrus in the camel is evinced by restlessness, excitement, bleating and wandering in search of the male. The vulval lips are swollen and there is appearance of slimy discharge.

On the first day of oestrus, the female tries to keep a close company with the stud. If let loose alone there is acute restlessness, animal remains off feed, tries to smell the male urine and homosexual tendency is noticed. There is frequent micturition and it does not allow its calf to suckle and rather leaves it alone during oestrus (Fig. 196 a to i).

### Post-partum oestrus

In a camel in good condition the first oestrus after parturition may occur within a month. In general, it occurs after a year but occasionally it may recur much earlier. In Somali Republic a number of females do exhibit oestrus just 6 months after parturition. Calf heat is usually manifested as foal heat in mares, 10 or 15 days after parturition or abortion but the fertility rate is as low as 2 per cent (Vyat and Pareek, 1969).

### Gestation period

The gestation period of the camel has been reported by several authors as under:



Mathur (1966)	370-390 days.
Mehta <i>et al</i> (1962)	Average $389.87 \pm 2.1$ days; for male calves $389.23 \pm 3.2$ ; for female calves $390.56 \pm 2.73$ ; for first calving $389.7 \pm 3.2$ ; for 2nd calving and for subsequent calvings $390 \pm 2.4$ days.
Sharma (1968)	Average $391.1 \pm 16.7$ days. Male calf $391.68 \pm 15.2$ days. Female calf $390.44 \pm 17.32$ days.

Investigations on 296 single humped camels, at Government Camel Breeding Farm, Bikaner showed the average gestation period as  $391.1 \pm 16.7$  days, males being carried slightly longer ( $391.68 \pm 15.2$  days) than the females ( $390.44 \pm 17.32$  days) which was not significant statistically. The sex of the calf and the calving sequence of the dam had no significant effect on gestation period but month of calving ( $P < 0.01$ ) and the sire of the calf ( $P < 0.05$ ) had a significant effect (Vyas and Pareek, 1969).

### Pregnancy Diagnosis in Camels

#### 1. PHYSICAL CHANGES DURING

PREGNANCY: (Vyas & Pareek, 1969).

- (a) *Cocking of tail*: The time taken by a pregnant camel to exhibit the sign of cocking of the tail is  $28.8 \pm 2.0$  days. However, this is not indicative of the stage of pregnancy.
- (b) *Body weight*: The pregnant camels showed a tendency for a higher body weight as compared to the non-pregnant.
- (c) *Colour of the urine*: The urine from pregnant camel showed yellow colour in 31.4 per cent, dark yellow colour in 48.5 per cent and light yellow colour in 5.7 per cent animals, whereas in

the non-pregnant camels the light yellow colour of urine was observed in 47.6 per cent and the rest voided yellow coloured urine. Dark colouration of the urine is an indication for pregnancy.

- (d) *pH of the urine*: The urine of pregnant camel is usually alkaline. As the stage of pregnancy advanced, pH of urine recorded was 8.0 as against the pH  $7.4 \pm 0.08$  recorded in non-pregnant camels. Therefore pH can serve as a good tool for the detection of stages of the pregnancy in camels.
- (e) *Specific gravity of the urine*: The specific gravity of urine samples of pregnant animals varied from  $1.038 \pm 0.010$  to  $1.086 \pm 0.003$ , whereas in non-pregnant urine samples, the specific gravity was  $1.036 \pm 0.00$ .
- (f) *Examination of vaginal vestibules*: In the pregnant camels the vaginal folds become prominent and found near vulva as the pregnancy advances presenting a false appearance of cervix, which is a peculiar phenomenon with this species. The mucous membrane of vagina is pale and sticky during pregnancy.



Fig. 196 (c) Showing female in oestrus sniffing external genitalia of male.



Fig. 196 (f) Female in oestrus showing face to male for contact with male.

Fig. 196 (i) Showing both male and female in oestrus finally coming close together.



Fig. 196 (g) Showing female in oestrus starting to come close to male.

Fig. 196 (h) Showing female in oestrus biting the muzzle of male while coming closer.



(g) *Anatomy and rectal palpation of the genitalia of the She-camel:*

The uterus is bicornuate and T shaped, resembling that of a mare, but the horns are not as wide apart as that of mare. The uterus is mainly in abdomen but extends in the pelvic cavity. The attachment of broad ligament is similar to that of cow and placement of the uterus is in the pelvic cavity as that of cow.

The pregnancy can be detected at 35 to 40 days of gestation by rectal palpation and can positively be confirmed at 60 days. The middle uterine artery can also be felt and the typical fremitus appears at about 80 days of pregnancy. The pregnancy corpus luteum is well developed at 60 days of gestation.

## 2. CHEMICAL TEST

Cuboni's test is good enough to detect early stage of pregnancy upto 60 days. Thereafter the test is unreliable. There is 80 to 90 per cent accuracy in early pregnancy diagnosis.

Barium chloride test showed 80 to 90 per cent accuracy at 50 to 60 days of pregnancy.

## 3. BIOLOGICAL TEST

Barlen (1969) considers the presence of gonadotropin like substances in the pregnant camel serum at 60 to 70 days of pregnancy.

## 4. VAGINAL CYTOLOGY

During pregnancy 3 types of cells predominate. The type I cells which are known as navicular cells predominate. These cells show typical folding and a large number of cells take yellowish stain due to high glycogen content in the cells. These changes are suggestive of pregnancy. The determination of

cell ratio is an aid to differentiate the type of cells.

It may be noted that the customary concept of the cocking of tail is the best and perfect method for detection of pregnancy and rectal palpation is extremely reliable to diagnose the stage of pregnancy. Amongst the auxiliary aids, the vaginal cytology is one of the best method, the Cuboni's test, pH and specific gravity of urine are recommended to further confirm pregnancy in camels.

## Intercalving period

Intercalving period is fairly fixed in the camel due to the golden rule of nature that half of the females in the herd are in calf while the remaining half are empty with calves at foot. Thus a camel cow goes empty every alternate year. Vyas and Pareek (1969) reported the average intercalving period as 751.7 days with a range of 716 to 771 days, the observations being based on 50 cow camels.

In case feeding standards are very good and if it is desired to obtain more calves, the first oestrus within a month after parturition has to be utilised for service. In India, breeders attempt to get their animals served in December or early January so that more time would be available for repeated services in case conceptions do not occur till March. During this period some males in rut would be still available for service.

## MALE

### Sexual maturity

The male may reach sexual maturity at three years and serve but his full reproductive capacity is not developed until the age of six years. Full grown male

may serve 50 females on an average in a year. A well fed male may cover upto 70 females in a season.

It is peculiar in the male camel that annually it has the period of sexual activity known as 'rutting period', which may last for a couple of months. For the remaining period of the year breeding instinct remains suppressed. The period of activity is seen from November to March when the day light is lengthening in the Northern hemisphere and from June to September in Southern hemisphere. At the equator, there is no definite rutting season and breeding activities may take place at any time of the year. The duration of 'Rut or Musth' in the male is largely dependant on the plane of nutrition. The average duration is about 3 months but it may last upto 5 months and occasionally in the old camels it may continue throughout the year.

#### **Behaviour of a stud camel during rutting season**

When a male camel is in Musth, he loses his appetite, often suffers from diarrhoea, lacks vigour, irritable, intolerant, rebellious and is preoccupied with an exhibition complex. Animal appears restless, mischievous and has intense sex desire. He tries to break all restraints and escapes from the enclosure and wanders aimlessly. An excited camel is difficult to control and even slight provocation causes great excitement. It produces gurgling sound with frequent throwing out of the soft palate from the mouth with the tongue on one side (Fig. 197). He may often swallow air like a wind sucking horse which he belches out. Camel in 'musth' constantly keeps on grinding his teeth and froths at the mouth. When

the air is belched out it draws along with it the soft palate which protrudes from one side of the mouth like a pink or pinkish black bladder and it remains visible for a few seconds to 2-3 minutes before it is drawn back into the mouth. This is associated with salivation. The animal becomes very ferocious and dangerous. During the rut period in the male from February to March, the soft palate increases in length and may be evaginated from the mouth (Char-ton, 1963).

The expiration is sometimes of a greater duration. The poll gland secretion is often noticed in drops falling on the neck. Leese (1927) and Purohit and Singh (1958) reported that the Poll gland is present only in camel and not in other species of domestic animals. These are two large cutaneous glands broad at the base and narrow at the apex situated in the skin below the apex of the head. These are found only in the males of the species each being about 9 cm. long, 7.5 cm. wide and 4 cm. thick with many excretory pores. In rutting season their secretion which is dark brown in colour with an offensive odour when much augmented, often runs down the neck. 'Before puberty and during the period between rutting seasons, these glands are inactive and consist mainly of connective tissue and alveoli scattered in groups of lobules. The camel in musth is sometimes seen standing with his legs apart, flapping his tail up and down and even emitting semen occasionally. The animal usually shows a marked pugnacity to other male camels and also the ones which are in musth. He usually bites at the withers, head, legs or scrotum. Camel bite is very severe. It can easily tear out strong muscle or take the top of ones head with ease.

Musth camels fall off in their conditions as the rutting season progresses. In some the fall in condition is to a perceptible degree. This is attributed to loss of appetite, wind sucking and diarrhoea during musth period.

When a camel is in musth it is advisable to tie his tail by means of a cord to the horn on the hump to guard against urine, faecal matter and semen which the camel splashes about from time to time.

A leather or net muzzle should be put on the face of such vicious camels. His usefulness during the rut period as riding or baggage animal is greatly diminished but if he is put to very hard work his sexual inclinations decline very rapidly. However it is risky to use the camel during musth period. In India it is very rare that the camel remains in musth for the whole year round. Such camels are dangerous and should be either castrated or destroyed.

A male camel which has been used for breeding for one season develops a fold of skin in the hollow of the hind pastern and also a bald thickening of the skin near the stifle. It is considered that 8 years is the best age at which the camel should be put to stud.

### **Mating behaviour**

The male camel may attempt to corner the dam as early as that of two years of age but sexual activity is not very well marked until six years.

Male in musth always appears ferocious. He carries his head high and keeps his hind legs apart with up and down movements of the tail. If there is any other musth or strange camel in view, fight often ensues resulting in

bites on the scrotum. During season from November to February the male camel will spot out females in heat. The she camel in heat will always prefer to remain near the male in musth and if she is forcibly ridden or loaded with baggage she will sit down. In this attempt to spot out the she camel in heat the male will move round the female several times and when he is sure that she is in heat he will usually put his neck on her neck and bite on her shoulder in his attempts to make her sit.

### **Posture at the time of copulation**

Copulation takes place in the recumbent position. The she camel sits normally while the male squats in the dog sitting position with hind limbs flexed and resting on the ground from the heels to the hocks, the stifle almost touches the ground, the fore limbs are extended on each side of the she camel with the feet holding strong to the ground to take firm hold of the female. Both the animals face the same direction (Fig. 198 a to h).

### **Intromission and ejaculation**

As observed by Singh and Prakash (1964) on introduction of the penis a sudden contraction of the abdominal muscles particularly of the rectus abdominis is noticed as a result of which the pelvic region of the male is quickly brought into direct apposition to the external genitalia of the female whereby complete intromission is accomplished. At this stage there is maximum frothing and gurgling noise from the male whereas the female grunts. Consequent to the complete intromission the male will make penile strokes as in the stallion. Semen is deposited in cranial part of vagina near the cervix.

### Copulation time

The copulation time recorded by Singh and Prakash (1964) during 31 services, shows maximum, minimum and average values as 20.0, 7.0 and 11.4 minutes respectively. When ejaculation occurs the muscles of the hind legs contract, there is accelerated respiration and the male stands up on his legs and comes off the female.

The sex act in camel appears to be a complex one. The marked sex drive, a short preparatory stages, co ordination and balance of the male during the act of mounting and grasping the female, attempts in search to locate the vagina and the penile strokes ending in ejaculation can be considered as special features of the complex act (Leonard, 1894; Leese, 1927; Singh & Prakash, (1964).

### Signs of pregnancy

In about 2 to 3 weeks time after the service, the she camel which has conceived *can be distinguished by the way she develops tendency to raise the tail upwards, whenever approached by a male or handled by a man.* It is considered as a sure sign of conception and it is also seen during next stages of pregnancy. If the tail hangs in the pendulous position, then she is taken as empty.

At about 6 months of gestation, the belly becomes enlarged but in the primipara the enlargement is not very marked. As the pregnancy advances, the vulva considerably increases in size and at parturition time it attains a length of about 18 cm. as against 6 cm. in non-gravid condition.

### Unequal function of reproductive organs and multiple births

Shalash and Nawito (1964) in their studies on 942 reproductive organs in

non-pregnant camels and 787 of pregnant ones obtained from the abattoir observed that 99.24 per cent of pregnancies were in the left horn and 0.76 per cent in the right. The incidence of cross pregnancy, twin pregnancy and triple pregnancy was 37.73 per cent. and 0.13 per cent respectively. The incidence of cross pregnancy in Somalia was found to be 40 per cent in 108 pregnant uteri investigated (Simon and Luktuke, 1977 unpublished data). Graafian follicles in the ovaries were observed in 4.82 per cent of the gravid organs. In 37.73 per cent gravid uteri, the foetus and the corpus luteum occurred on the opposite sides probably indicating migration of the fertilized ovum. Ovarian activity was 12.92 per cent higher in the left than in the right ovaries. Multiple mature Graafian follicles were found in the ovaries in 43.8 per cent of gravid cornua. Highly significant ovarian activity was noticed between months and between seasons. The percentage of functioning ovaries was 19.39 in winter, 27.48 in spring, 6.66 in summer and 6.88 in autumn. The number of services per conception was 1.2 and the cases of sterility, repeat breeders and genital dysfunctions were very few.

### PARTURITION IN CAMEL

The usual signs approaching parturition are swelling of the vulva, enlargement of the udder and sinking of the pelvic ligaments forming a groove on either side of the tail. The she camel is secured in sitting position by tying the forelimbs with help of a string. The forefeet appears first followed by the head lying in between them and they should be secured when labour pains start. The duration of labour pain is more in camel than in



Fig. 198 (c) Male taking mounting stance.

198 (f) Copulatory action.  
Male extends fore  
limbs.

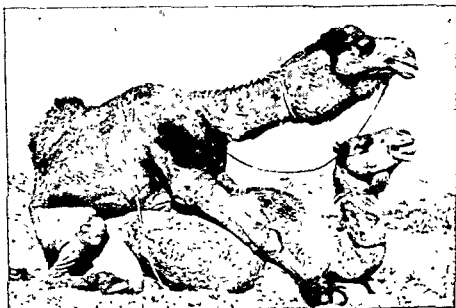


Fig. 198 (g) Camel in copulatory  
orgasm.

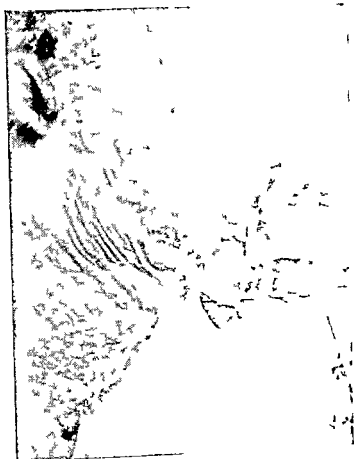


Fig 197 Camel in Musth throwing out the soft palate with tongue on one side

Fig 199 PARTURITION IN CAMEL





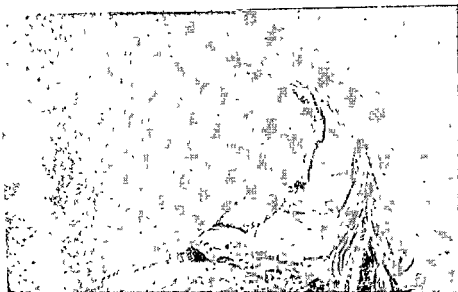


Fig 199 (b) Parturition in camel.  
Note the mucus discharge.

Fig 199 (c) Parturition in camel.  
Appearance of water-bag

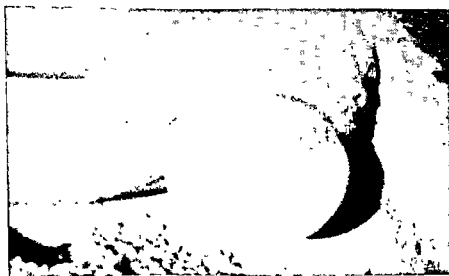
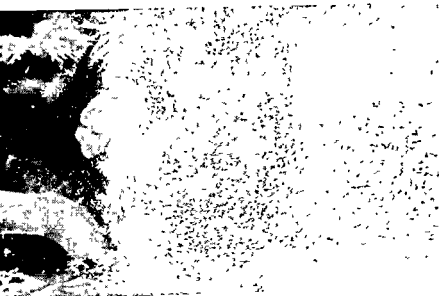


Fig. 199 (d) Parturition in camel.  
Chorio-allantoic sac  
just before rupture.



199 (f) Parturition in camel.  
Appearance of head  
and fore limbs of calf  
upto knee joint.

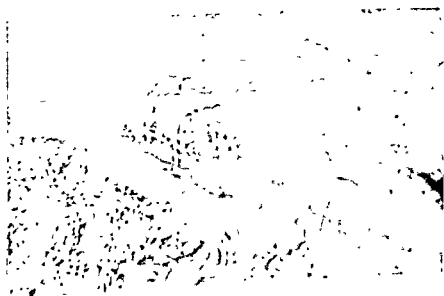




Fig. 199 (h) Parturition in camel. Appearance of thorax, abdomen and hind parts of calf.



Fig. 199 (i) Parturition in camel. Calf just after birth.



Fig. 200 (a) She camel after parturition showing placental sac coming out.



Fig. 200 (b) She camel just after parturition showing stump of foetal membranes hanging from vulva.

cow. The parturition takes place in sitting position and the umbilical cord ruptures when the dam rolls to lick the young one. Foetal membranes are expelled soon after the foetus is born. Prakash and Singh (1962) observed marked swelling of the udder and teats in the first stage with oedema of the vulva and relaxation of sacrosciatic ligaments. The animal exhibiting such signs should be secured otherwise it keeps on running till calving takes place. Rumination becomes irregular, tail is raised and animal looks at the flank because of colicky pains. The average duration of labour reported is 5 hours 36 minutes with the maximum of 10 hours. After appearance of water bag, she camel adopts a side posture with extended limbs to expell the foetus (Figs. 199 a to i). Prakash and Singh (1962) recorded in 45 camels the minimum, maximum and mean duration of labour pains as 120, 600 and 366 minutes respectively ( $SD \pm 139.3$ ).

In most cases foetuses are presented in anterior, longitudinal presentation and dorsosacral position, with the chin resting on carpus (Sharma and Vyas, 1970).

The average time taken for different stages of parturition is as follows (Table 83).

The she camel remains recumbent after delivery for 1 to 39 minutes (average for 7.3 min.). The camel calf attains standing position in 204.3 minutes (37 to 394 minutes) after birth. The umbilical cord ruptures at a length of  $25.01 \pm 3.77$  cm. from the umbilicus (Fig. 200 a, b).

The average weight of foetal membranes is  $6.31 \pm 1.6$  kg. The weights of the foetal membranes of male ( $6.40 \pm 1.47$  Kg.) and female ( $6.29 \pm 1.38$  Kg.). Camel calves do not differ significantly but there is a positive correlation between weight of the calf and the weight of the foetal membranes.

Prakash and Singh (1962) recorded average time of 117 minutes for the expulsion of foetal membranes with minimum and maximum as 65 and 330 minutes respectively. The average total time required for expulsion of foetal membranes of male and female calves is  $152.34 \pm 97.50$  and  $121.57 \pm 61.5$  minutes, the average being 117.8 minutes

Table 83  
TIME REQUIRED FOR DIFFERENT STAGES OF PARTURITION IN THE CAMEL

No. of animals	Primipara 12	Pluripara 51
Duration of 1st stage (Minutes)	$168.0 \pm 51.6$	$199.50 \pm 99.20$
Duration of 2nd stage (Minutes)	$105.0 \pm 4.65$	$84.1 \pm 4.91$
Duration of 3rd stage (Minutes)	$109.90 \pm 49.62$	$146.13 \pm 91.90$
Average time required for complete process of parturition (Minutes)	$281.81 \pm 79.62$	$355.90 \pm 79.30$

Out of 63 calvings there was retention of placenta in seven (Sharma, 1968). Caesarean operations have been reported to be quite successful in she camels in order to relieve dystokia (Petrís, 1956; Rathor, 1962; Sharma and Pareek, 1970).

### **Involution of uterus**

The average interval between parturition and complete involution of uterus in she camels is  $40.20 \pm 2.10$  days, ranging from 31 to 54 days (based on 30 observations). The involution rate indicated by haematological studies showed a rapid involution in the first 15 days post-partum which considerably becomes slower during the next 3 weeks to follow. Involution is fast in animals calved for the first time (34.14 days) but it is delayed during the subsequent calvings. The neutrophils and lymphocytes increase with the advancement of involution of uterus while monocytes decrease and basophiles remain constant. A day after parturition, there is sudden increase in neutrophils followed by rapid decrease, the opposite being for the lymphocytes and by the time involution is complete the lymphocytes, neutrophile ratio becomes 1:2.

The vulva returns to its normal size within about 31.7 days (16 to 42 days). The average length of vulva from parturition to four days post-partum is 9.1 to 17.8 cm. while near the end of involution it is 6 to 7.1 cm.

The lochial discharge during puerperium is noticed to be dark red to light red in colour which ceases after complete involution (Vyas and Pareek, 1969).

### **Sex Ratio**

Bhargava *et al* (1963) reported the secondary sex ratio in Bikaneri camels

as 97.11:100 out of 205 normal births (101 males and 104 females). Shalash and Nawito (1964) reported a sex ratio of male and female calves as 47.48:52.52 in 516 births.

### **Birth weight**

The birth weight of Bikaneri camel as reported by Bhargava *et al* (1963) was  $81.1 \pm 0.82$  lb. Male had significantly higher birth weight ( $84.2 \pm 0.3$  lb) than the female ( $82.0 \pm 1.02$  lb). The average birth weight of males was  $39.7 \pm 5.2$  kg. significantly being heavier ( $40.8 \pm 5.7$  kg.) than the females ( $38.4 \pm 4.5$  kg.) Calving sequence, sex and month of freshening had a significant effect on the birth weight of the calf. There is a positive correlation between gestation period and birth weight of camel calf.

### **Reproductive span**

The female can produce on an average 6 to 10 calves in her life time but after about 18 years of age, general debility sets in and the calves that are born afterwards are weak and poor in condition. In some cases they maintain their breeding efficiency upto the age of 20 years. However, Yaseen and Vahid (1957) from Pakistan reported that reproductive span may extend upto 30 years.

### **Rearing**

Colostrum should sparingly be fed to young one, since it has relatively more laxative effect. The first 20 days period of life is a critical period in the life of the new born and as such care is taken that it is not overfed with milk. Usually only one teat is allowed to the young one and the remaining three are milked out by hand twice a day and the teats tied up with a string. Diarrhoea

and death may occur in case the young one gets an access to suckle heavily.

Upto about a week or so the young camel is unsteady on its legs. In about 10 days time it is fit to move with the mother when she is left for grazing. After about 20 days when the critical period has passed, the young one may be allowed to suck two teats for the next two months or so, then three teats for the next three months and at about six months, it may be allowed the whole udder. In case the mother is fed only on dry forage with little or no grazing then it will be necessary to give the calf all the milk even much earlier.

The she camel is milked twice a day for the first two weeks after delivery. The daily yield of milk from a fairly good dam is about nine litres with an average of 2727 litres in a lactation. The lactation period is dependant on the quality of grazing and season. In the desert tract the female may dry up in about eight months time while as in good grazing areas she may continue her lactation for the period of 18 months or even longer to 2 years. It is best to wean the calves at 15 months if they are still suckling.

If the dam dies within 3 months after the parturition, it is difficult to rear the young one. If over 3 months of age, it can readily be fostered on another she camel. It can also learn to suck milk from a spouted vessel.

The young ones of camel begin to graze between 4 to 6 weeks.

Even if the young one dies, in majority of cases the female may continue to give milk. In difficult cases letdown may be induced by presenting before her a dummy calf with the skin of the dead.

## Camel milk

Camel milk is opaque, white in colour with a milky odour and thinner in consistency than that of the cows milk. It has got a salty taste with an average fat percentage of about 3 ranging from 2.6 to 4.3. The specific gravity is 1.025 to 1.0278 with an average of 1.0270.

## Milk yield

The average milk yield worked out at the camel breeding farm by selecting twelve cow camels randomly found to be 5.18, 6.27, 6.36, 6.18 and 5.13 (lbs) at 1st, 2nd, 3rd and 4th weeks and 2 months after parturition, respectively.

## Reproductive disorders

Very little is known about the reproductive biology of camel and hence it is necessary to undertake further research to probe into causative factors leading to infertility. Yet it can be said that the faulty management, uncontrolled breeding and disease factors can be attributed as the chief reasons responsible for infertility. Out of the scanty literature available in this respect, some work has been reported by Shalash and Nawito (1963) and Bhargava *et al* (1963). They recorded the partial as well as complete hypoplasia of ovaries, subfunctional ovaries, ovarian tumours, cystic ovaries, dermoid cysts and haemorrhagic cysts in the ovaries. Cystic corpus luteum, bursal adhesions, patent os uteri, infantile uteri, cysts and abscesses of uterus, haemorrhagic endometritis, pyometra, foetal maceration and mummification, embryonic mortality, incomplete development of the uterus and persistent hymen.

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A high rate of abortion in she camels mostly during the latter half of the pregnancy is attributed to Trypanosomiasis (Surra).

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